INVESTIGATION OF DNA SEQUENCE-DIRECTED REVERSIBLE SWELLING OF DNA-CROSSLINKED HYDROGEL

By

Yixin Liu

A thesis submitted to Johns Hopkins University in conformity with the requirements for the degree of Master of Science in Engineering

Baltimore, Maryland
May 2021

Abstract

Stimulus-induced shape change of hydrogels is a technology critical to the development of many soft microscale robots. As a result, the study of stimulus-induced hydrogel shape change has attracted substantial interest from researchers in diverse fields, especially in biomedical research. The shape change induced by DNA molecules with specific sequences, unlike the shape change induced by nonspecific stimuli (UV light, pH, and temperature), can be highly selectively controlled, allowing control of hydrogel swelling akin to the control achieved over electronically controlled robots. DNA-crosslinked hydrogels can recognize and respond to specific these sequences of nucleotides and can be copatterned to assemble complex devices; this technology shows great potential for building smart machines.

In this thesis, a novel DNA-controlled reversible swelling hydrogel system was investigated. Hairpin-shaped DNA single strands are inserted into DNA crosslinks through strand displacement reactions sequentially to elongate the crosslinks, and the size of the hydrogel increases after this addition. The inserted hairpins can hybridize with reversals to be removed from the crosslinks to realize contraction in size.

To better understand the swelling process, we designed mass balance experiments to measure the amount of DNA hairpins added to a hydrogel during swelling and removed during hydrogel contraction. This measurement was performed by measuring the concentration of the hydrogels remaining in the solution at different points during swelling and then calculating the hydrogel's hairpin intake as the decrease in the concentration remaining in solution over time. Comparing the rates of hairpin intake and the rates of hydrogel swelling allowed us to conclude that 1) the

concentration of DNA crosslinks in the gel have an enormous influence on the speed of hairpin uptake and the equilibrium extent of swelling, 2) higher DNA hairpin concentrations lead to a larger final hydrogel size, and 3) the osmotic pressure has an impact on the strand displacement reaction.

We also applied the Maxwell model of polymers with the goal of understanding why 1) the degree of swelling declined slightly with the number of reaction cycles, 2) more significant swelling results in a less elastic polymer with the same system and concentrations of DNA crosslinks, and 3) the time lag between hairpin intake and hydrogel size change. These ideas will help us better understand the process of reversible swelling of DNA-crosslinked hydrogels and suggest means to improve its speed and efficiency during further studies.

Advisor: Dr. Rebecca Schulman

Reader: Dr. David Gracias

Acknowledgements

During my two years of master's research, I received generous help from many people. First, I would like to express sincere gratitude to my advisor, Prof. Schulman, for her instructive advice and insightful suggestions on my research. Besides that, she also taught me a lot about the fascinating DNA nanotechnology. I would like to say thank you to Rachel. She showed great patience in teaching me how to do the experiments and gave me valuable suggestions on my experiments.

Moreover, I am grateful to Lei, Charlie, Zach, Misha, and other lab members. From the discussion and collaboration with them, I learned a lot. Finally, I would like to thank my family. It is their support and encouragement that inspired me to study and work hard.

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1 Introduction

1.1 DNA

Deoxyribonucleotide (DNA) has received considerable attention since the Watson-Crick model for double-strand helical DNA was developed. A single-strand DNA molecule is a polynucleotide composed of a monomer named nucleotide[1]. A nucleotide is made up of one five-carbon sugar molecule linked with one of the four nucleobases (A for Adenine, T for thymine, C for cytosine, G for guanine) by a glycosidic bond and a phosphate group. Since stable hydrogen bonds form between A and T and between C and G, a single strand DNA can combine with another single strand, the sequence of which is complementary to the first strand. Thus, a simple but elegant helix structure of one double-strand DNA is formed[2]. This nature-evolved molecule can encode and express genetic information through the base pair sequence and conserve its original sequence.

The highly selective and reversible affinity to the complementary strand is the basis for DNA nanotechnology[3-5]. Besides the critical role of DNA in life science, it is also a prominent character in material science and many other fields. Take DNA origami as an example. A carefully designed single strand can induce the self-assembly of DNA on the molecular scale, thus finally controlling the local structure in a complex[6-8].

One other advantage of DNA is that it can be easily programmed and functionalized. The programmable trait of the DNA sequence creates a vast space for strip design[9]. A DNA-functionalized ligand can introduce DNA to a metallic particle with a relatively solid coordinate bond and show interesting phase behaviors[10-12]. Linking DNA with acrydite moiety offers a simple way to synthesize polymers with DNA in its polymer structure[13-14].

1.2 Versatile Responsive DNA-related hydrogels

1.2.1 Responsive hydrogels

Hydrogels have gained more and more popularity in the past several decades because of their biocompatibility and responsive behavior in variable surroundings. Two common strategies of synthesizing responsive hydrogels are 1) using stimuli-responsive polymers and 2) adding crosslinks or stimuli-responsive functional groups to the polymer structures.[15] For hydrogels synthesized based on these two strategies, changes in the surrounding environments may cause conformation transition of the polymer chains, which turned out to be a visible swelling or a significant change in mechanical properties.[15]

External stimuli-induced shape change of hydrogels is critical to the design of the soft robot and there has been substantial interest in the use of these hydrogels in material science and biomedical research. The shape change induced by DNA sequence, unlike the nonspecific stimuli (UV light, pH, temperature, and salt concentration), can be highly selectively controlled, mimicking the wired systems[16]. DNA-crosslinked hydrogels that can recognize and respond to specific sequences of nucleotides could be incorporated together and show great potential in building smart machines.

1.2.2 DNA hydrogel

Several types of hydrogels have been constructed using DNA molecules or DNA molecules combined with other polymer materials. Y-shaped DNA and DNA duplexes have been used as building blocks of self-assembled DNA hydrogels[17-19]. Y-shaped DNA connects connected by DNA linkers can form a 3D network structure[17,19]. Such a DNA hydrogel without any backbone is sensitive to many stimuli and can significantly change mechanical property when stimulated.

DNA hydrogel of a particular shape synthesized through rolling circle amplification and multi-primed chain amplification had a liquid-like property without water and a solid-like property with water. The solid-like DNA hydrogel can keep its original size and shape after several rounds of removing and reintroducing water.[17]

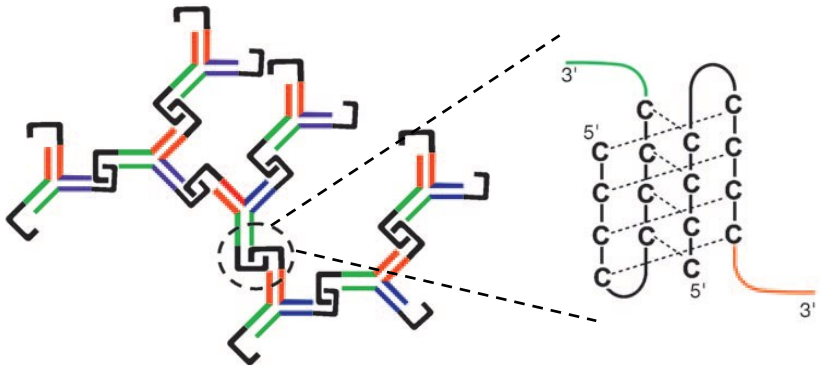

Figure 1 Diagram of pH-responsive hydrogel[18]

DNA hydrogel synthesized with cytosine-rich Y-shaped DNA (Figure 1) was sensitive to pH. At a low pH, the cytosine in cytosine-rich strand could be partially protonated and form a C....CH^+ triple hydrogen bond between these strands. Solution with controlled pH can reversibly turn the solution into a hydrogel. DNA hydrogel with this property had a great potential in the control delivery and release of drugs[18].

Some bio-molecules can also trigger mechanical property changes. The hydrogel formed by carefully-designed Y-shaped DNA and line-shaped aptamer linker can serve as a Thrombin detector. Thrombin combined with aptamer and broke the hydrogel to release the negatively-charged Au nanoparticle entrapped in the hydrogel network, which would quench the positively-charged quantum dots in the outside solution. The sensitive change in fluorescence could be used for detecting as low as 67nM Thrombin in the solution[19].

1.2.3 DNA-crosslinked hydrogel

Double strand DNA usually plays the role of crosslink in DNA-crosslinked (as opposed to

DNA) hydrogels, while polymer chains, like PEGDA, Acrylamide, or CMC, play the backbone's role[16,22,24,26]. Properties such as stiffness, size, and the rate of release of other signals generally of DNA-crosslinked hydrogels changes can change in response to stimuli[22-24]. One common method of inducing a response of a DNA-crosslinked hydrogel is to use a single-strand DNA molecule that can combine two other single strands of DNA that are conjugated to the polymer network. The stiffness of the hydrogel increases because such a binding reaction forms new crosslinks[21]. This change of stiffness can be made reversible process if the single stranded DNA inducing the change has a toehold[22]. Another single strand of DNA can then hybridize with the toehold strand to actuate a strand displacement reaction. The third strand hybridizes with the fourth strand and the resulting duplex leaves the hydrogel. The hydrogel's polymer structure goes back to its original state and can be restimulated by another addition of the third DNA strand.

A change in stiffness or size of a DNA-crosslinked hydrogel can be utilized in soft robots. An example of this idea is a soft "robot" with five fingers, where each finger bends in response to a specific DNA signal. Such a robot can make multiple gestures, including holding and taking stuff[23]. (Figure 2)

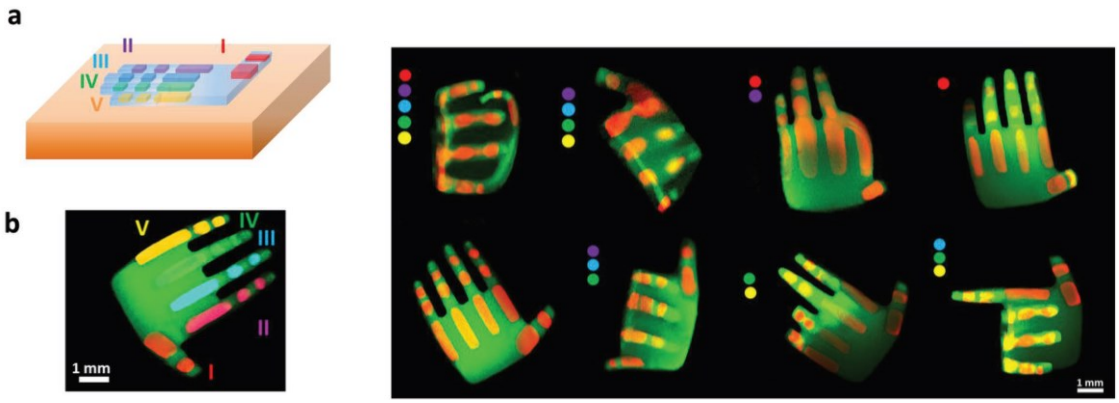

Figure 2 Control the movement of hydrogel palms with combined DNA trigger. a) Representative fluorescent image of a hydrogel palm containing five different Ls at desired location. c) Eight gestures obtained from DNA trigger induced movement.

UV light is also capable of inducing changes in hydrogels. The azobenzene-tethered single-strand DNA hybridizes to its complementary strand in its trans- configuration but cannot hybridize in its cis- configuration[24]. The trans- configuration is favored under UV light while Cis- is favored under visible light, so switching the light will significantly influence the hybridization and the gel's stiffness. Reversible drug control release within ten minutes can be achieved based on this idea[25]. A third azo-modified single-strand DNA (strand A-B) is added to a mixture of two DNA polyacrylamide conjugates (complementary to A and B, respectively) to form a hydrogel. When UV light is applied, the azo-modified strand no longer hybridizes with the DNA polyacrylamide conjugates. The gel undergoes a gel-sol transition, at which time all the molecules trapped in the hydrogel diffuse out.

A hydrogel with DNA crosslinks and donor-acceptor crosslinks can be responsive to both DNA strands, affecting the DNA crosslinks, and oxidizing reagents, which turned donor-acceptor crosslinks into acceptor-acceptor repulsion[26]. This kind of design will improve the performance

and add new functions to DNA-crosslinked hydrogels.

1.3 DNA stimulated high-degree swelling of DNA-crosslinked hydrogel[16]

As Figure 2 delineates, the combination of several different DNA systems in one gel enables it to identify different DNA strand signals and work independently simultaneously. It is promising in the design of soft robots if the performance can be improved. It is published that numerous DNA strands can be inserted into DNA crosslinks and thus massively elongate the DNA crosslinks. (Figure 3)[16].

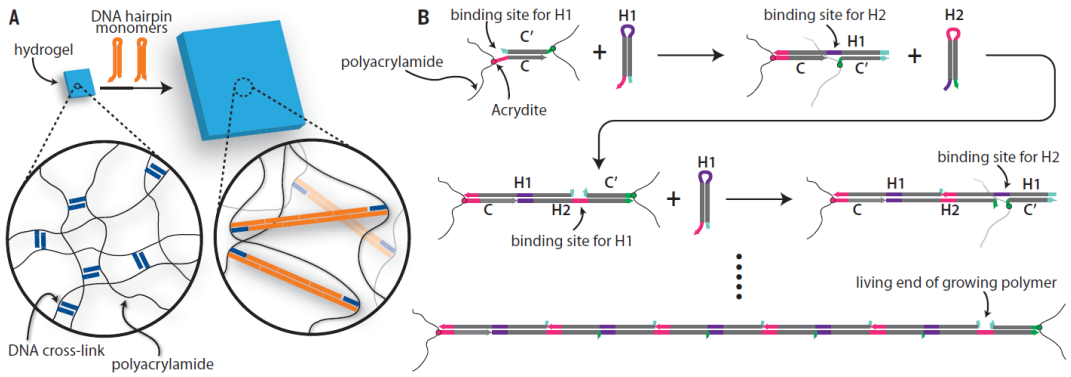

Figure 3 DNA-directed expansion of DNA–crosslinked polyacrylamide gels. (A)Swelling of DNA–crosslinked polyacrylamide hydrogels (B). Hairpins can insert into crosslinks, inducing hydrogel expansion. Colors indicate domain type and its complement. Thin black lines indicate polyacrylamide.[16]

For that purpose, two complementary toehold strands are designed on the DNA crosslinks and DNA strands. The DNA toehold strand will hybridize with the binding area on DNA crosslinks to trigger the strand displacement reaction[27-29]. After that, the hairpin is inserted into the crosslink. While still there is an unhybridized area on the DNA strand, waiting for another hairpin to insert. The added DNA strands are in the shape of a hairpin to avoid unwanted hybridization between DNA strands. Such exquisite design enables the two kinds of DNA

hairpins to take turns getting involved in DNA polymerization and swell the hydrogel.

1.4 Scope of this thesis

We can easily see the promising future where DNA-crosslinked hydrogels can be a practical part of soft robots from the above introduction. In this thesis, we studied a DNA-controlled reversible swelling hydrogel system. The PEGDA10k hydrogel and Acrylamide (with 5% BIS crosslinks) hydrogel with this design were synthesized based on a published method. With the investigation of swelling and reversing experiments going deeper, we found some factors that had an impact on the equilibrium degree of swelling and reversing. To better understand the swelling process, we measured the DNA hairpin intake of the gel during swelling. A theoretical model was introduced to explain some phenomena observed during the swelling.

2 Material and Methods

2.1 DNA sequence design and DNA stock preparation

2.1.1 Strategy of DNA sequence design

Investigations were performed in a system in which acrydite-modified DNA oligonucleotides served as crosslinks and two sets of DNA hairpin, reversal, and terminator hairpin were designed to meet the following requirements (Figure 4):

(1) The two acrydite-modified DNA oligonucleotides (A1: a'b' and R1: ybx. Lowercase letters in Figure 4 indicate different DNA sequences. While lowercase letters with a quote mark indicate the complementary strand to those DNA sequences) can hybridize with each other but leave an unbound area to enable the insertion of DNA hairpins.

(2) The first DNA hairpin(hairpin1) has a toehold strand(a) that can hybridize to unhybridized region of A1, allowing a strand displacement reaction to occur in which the hairpin will interact with both two DNA crosslinks and become inserted into the polymer chain while still leaving an unhybridized part(c) for the hairpin2 to insert. hairpin1 has a long tail(m) that does not hybridize with any of the hairpin, crosslinks, and terminator hairpin. This domain is used when hairpin1 reacts with reversal strands that direct the removal of the hairpin from the chain as described below.

(3) hairpin2 reacts with hairpin1 and crosslink2 once hairpin1 is inserted into the crosslink but leaves the unhybridized strand(a') for hairpin1 to insert. A long tail is also required on hairpin2(n).

(4) A terminator hairpin, a variant of each hairpin, will combine with the hairpin and the crosslink but leave no unhybridized parts. If the terminator hairpin is inserted, no other hairpin can perform the strand displacement reaction anymore. The design of terminator hairpins aims to prevent the infinite insertion of hairpins and stop the swelling when gels reach a preferable size.[16]

(5) The reversals will react with hairpins by hybridizing at their long tail such that the reaction removes the DNA hairpins from the polymerized crosslink. The two reversals will not hybridize with each other.

(6) Any secondary structure interactions not explicitly designed should be minimized. This minimization was achieved in sequence design using the DNA design package from NUPACK. If several different DNA systems (crosslinks, hairpins, reversals and terminators) are needed, they must be orthogonal to (ie have minimal secondary structure interactions with) each other.

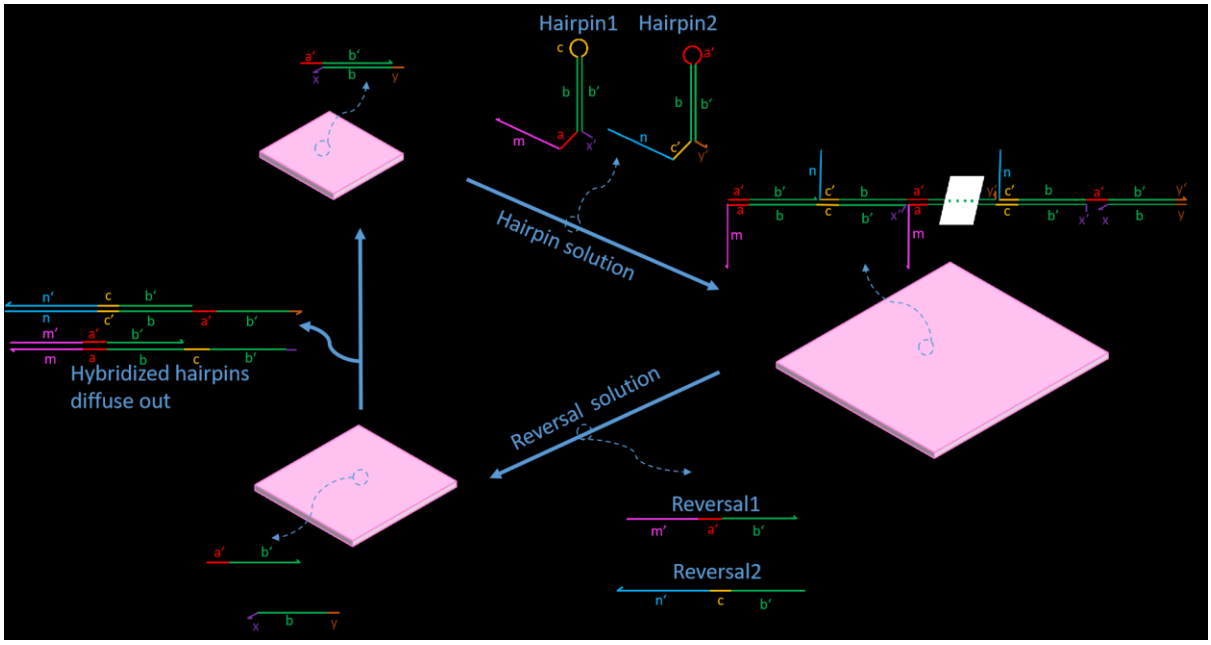

Figure 4 DNA-directed reversible swelling. Colors indicated different DNA strand and its complementary strand. Black line indicated the polymer chains.

Thanks to our lab member, Kuan-Lin Chen's hard work, four such systems were designed and available for use. A multi-domain hydrogel that can respond differently to different DNA hairpins can be designed and synthesized. Detailed DNA sequences are given in the appendix. All the DNAs were supplied by IDT in their lyophilized form.

2.1.2 Preparation of DNA stock.

DNA crosslinks were used in 1×TAE/12.5×10^{-3} M Mg^{2+} buffer: 1×TAE buffer was diluted from 50× stock, Life Technologies, #24710-030; magnesium acetate tetrahydrate, Sigma #228648) buffer is added into the DNA tube based on the weight of lyophilized DNA from IDT to get a 15mM solution of acrydite-modified DNA. After the DNA is dissolved in this buffer at room temperature, 1μL of the solution is removed and diluted with 100μL

1×TAE/12.5×10^{-3} M Mg^{2+} buffer. 2μL of this new solution is moved into a cuvette and diluted 100 times with 1×TAE/12.5×10^{-3} M Mg^{2+} buffer. Then it is mixed with pipettes several times, and a biophotometer is used to test the absorbance at 260nm. Given the DNA strand's extinction coefficient and the light absorbance, a more accurate concentration of the DNA crosslink stock can be achieved.

$$\text{DNA crosslink concentration}(in\ \text{mM}) = \frac{A\text{bsorbance at 260nm}}{\text{Extinct coeff}} \times 10100 \times 10^3$$

For the hairpins, reversals, and terminators, a certain amount of MilliQ water is added into the DNA tube based on the weight of lyophilized DNA from IDT to get a 4mM hairpin solution. After dissolving, 1μL is moved out and diluted with 50μL water. Then 2μL of the diluted solution is moved to a cuvette and diluted with 198μL water. A more accurate concentration is calculated based on the absorbance at 260nm from the biophotometer and the extinct coefficient.[30]

$$\text{DNA hairpin concentration}(in\ \text{mM}) = \frac{A\text{bsorbance at 260nm}}{\text{Extinct coeff}} \times 5100 \times 10^3$$

2.2 Synthesis of hydrogel

2.2.1 Preparation of photolithography chamber

The process of preparing a photolithography chamber is based on a published protocol from Professor David Gracias[31]. The photolithography chamber contains two parts. One is a clean glass slide at the bottom to which the hydrogels adhere after UV exposure. The other is a chromium mask at the top, which can selectively allow UV light to go through it in areas of a specific shape (squares with side length of 1 mm in this thesis). Only the pregel solution in the exposed area will polymerize and form hydrogels. To prepare the chromium mask on the top, briefly, a clean glass slide is coated with the photoresist SC 1827 (Microposit S1800

Series) by spin coating, followed by curing and UV exposure under a printed 2D mask. Then physical vapor deposition is applied to coat the slide with chromium. The slide is then rinsed with acetone and isopropanol, followed by drying under nitrogen gas to remove the positive photoresist in the unexposed area. Finally, it is spin-coated with CYTOP (Type M, Bellex International Corp.).

One single layer of polyimide tape is placed on the bottom slide as a spacer of approximately 160 μm. The side length of the square-shape hydrogels synthesized based on this method is 1mm. The height of the gel is approximately 160 μm.

2.2.2 Preparation of pregel solution

The method to synthesize PEGDA10k hydrogels and Am-5Bis hydrogels was previously reported by Shi et al. [30]

Two solutions of acrydite-modified crosslink (A1 and R1) are first brought to room temperature. Then 3μL of A1 and 3μL of R1 are transferred into one 200μL tube and diluted with 9μL 1×TAE/12.5×10^{-3} M Mg^{2+}. The final concentration of each crosslink is 3mM. This mixture needs to be annealed at 90℃ for five minutes, followed by cooling from 90℃ to 20℃ at 1℃/min to hybridize.

To make DNA-crosslinked polyacrylamide gels, an acrylamide solution was prepared at a concentration as 1.41 M. This concentration is kept the same in all Am-5Bis gels we synthesized in this thesis, where a 40 wt% acrylamide monomer stock was made using acrylamide from BIO-RAD #161-0100 and MilliQ water. The BIS concentration was varied by adding a 100×10^{-3} M BIS stock solution made from an N, N′-methylenebis(acrylamide) powder (Sigma-Aldrich, #146072) to make Am-5×10^{-3} M BIS-DNA. The concentrations of

the other components in the pre-gel solutions were: 1.154×10^{-3} M of annealed DNA crosslinks, 3% v/v Omnirad 2100 (formerly known as Irgacure 2100, IGM Resins USA, #55924582) photoinitiator (previously made to a 75% volume/volume butanol solution), and 2.74×10^{-3} M methacryloxyethyl thiocarbamoyl rhodamine B (Polysciences, Inc., #23591) fluorescent dye in 1×TAE/12.5×10^{-3} M Mg^{2+} buffer.

	Mass	**Stock**	**Final**	**Volume**
Water				11.9101356μL
TAEM		10×	1×	3μL
DNA Crosslink		3mM	1.154mM	11.54μL
PEGDA10k	3mg	100(w/v%)	10(w/v%)	
Rhodamine Methacrylate		29.93mM	2.74339mM	2.74986μL
Omnirad 2100		75(v/v%)	2(v/v%)	0.8μL
Final Vol				30μL

Table 1 Recipe for PEGDA10k DNA-crosslinked hydrogel

After mixing with pipettes thoroughly, the mixture is sonicated for 1 minute and degassed for 15 minutes under vacuum.[30] To make DNA-crosslinked PEGDA hydrogels, 3mg PEGDA10k powder (Sigma-Aldrich, #729094) is weighed and transferred to a 200μL tube after warming up at room temperature. To prepare a pregel solution with 1.154mM crosslink, water, 10× TAE/Mg^{2+} buffer, annealed DNA crosslinks, 30 mM methacryloxyethyl thiocarbamoyl rhodamine B stock solution, and Omnirad 2100(v/v butanol=75%) is then added into the tube according to the recipe in Table 1.

Other procedures were the same as the synthesis of Am-5Bis gels, except that after mixing

with pipettes thoroughly, the mixture is sonicated for five minutes and degassed for 15 minutes under a vacuum.

2.2.3 Photopatterning of pregel solution

The pregel solution is dropped onto the chromium side of the chromium mask. The bottom slide with a spacer is placed on the mask after that. The chamber is assembled by clipping the two slides together with binder clippers and then exposed to 365nm UV light for a total light dose of 800mJ/cm^2 for PEGDA10k gel and 150mJ/cm^2 for Am-5Bis gel.

After the photopatterning, the chamber is opened, and most gels stick to the chromium sides. They can be moved from the slides with tweezers after rinsing with 1×TAE/12.5×10^{-3} M Mg^{2+} buffer. They should be soaked in the buffer solution in a gel plate, sealed, and kept in the refrigerator at 4℃ for at least one day to get fully hydrated before using.

2.3 Preparation of solutions in swelling and reversing experiments

2.3.1 Preparation of hairpin solutions in swelling experiments

Take System 5 hairpins as an example. System 5 hairpin1 and terminator hairpin1 are moved from the stock into two 600μL tubes and diluted to 400μM solution with 1×TAE/12.5×10^{-3} M Mg^{2+}. (Table 2), which contains 1% of the terminator hairpins. (396μM hairpin1 and 4uM terminator hairpin1)

400μM System 5 hairpin2 solution is also prepared based on the above protocol.

It is a prerequisite that all the hairpins are folded before use for hydrogel actuation. So, the two 400μM hairpin solutions should be heated to 95℃ for five minutes and kept for 10 extra

minutes. After that, the two solutions are cooled in an ice-water mixture immediately. Finally, 270μL of the 400μM hairpin1 solution and 270μL of 400μM hairpin2 solution are added to 1260μL 1×TAE/12.5×10^{-3} M Mg^{2+}(0.01% Tween) buffer and mixed well to get a 1800μL solution (0.007% Tween) with 60μM hairpin1 and 60μM hairpin2.

				% Terminator	**1**
	Identifier	**Stock (mM)**	**Final (μM)**	**Dil Factor**	**Volume**
1xTAEM		1			252.9091896
S5H1R	292531957	3.125386373	396	1	36.74425696
S5_HP1term	262271525	3.347247014	4	1	0.34655345
Total					290

Table 2 Recipe for 400μM DNA hairpin solution

2.3.2 Preparation of reversal solutions in reversing experiments

We describe the protocol for preparing reversal solutions using the System 5 reversal strands as an example (See Appendix for the sequences of these strands). Stock solutions of system 5 reversal1 and hairpin2 are each diluted with 1×TAE/12.5×10^{-3} M Mg^{2+} to produce 2 solutions that each of a final concentration of 400μM reversal of one of the reversal strands. (Table3)

Then 140μL of both of these solutions are combined to form a solution in 653μL 1×TAE/12.5×10^{-3} M Mg^{2+}(0.01% Tween) buffer with final concentrations of 60μM reversal1 and 60μM reversal2.

				% Terminator	**0**
	Identifier	**Stock (mM)**	**Final (µM)**	**Dil Factor**	**Volume**
1xTAEM		1			125.0770105
RevH1_Sys5	283161807	3.752599307	400	1	14.92298949
Total					140

Table 3 Recipe for reversal solution

2.4 Process of the swelling and reversing experiments

2.4.1 Process of swelling experiments

150µL of 60µM hairpin solution is pipetted into one well of a 96-well plate. Then one fully hydrated hydrogel is picked up with tweezers from the gel plate and lay flat onto the bottom of that well. We used a camera to capture images of the hydrogels (Figure 5) that was set up by KuanLin Chen.

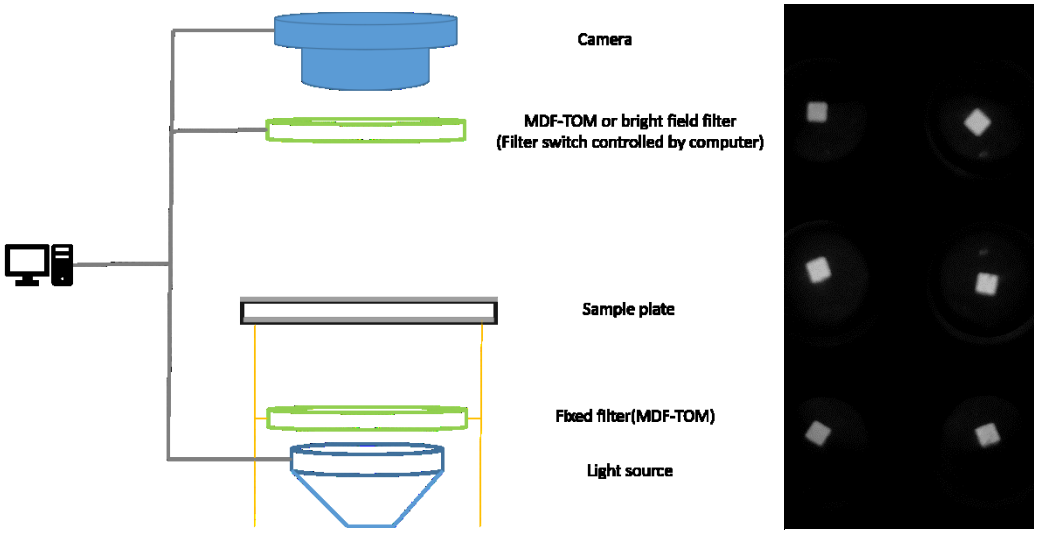

Figure 5 Scheme of Pi Imager, the photo-taking device and a photo example

To record changes in size of hydrogels, one photo is taken every 30minutes for 100h (time of exposure is 5s). MATLAB code further processes the times series photos to analyze the side length. (The MATLAB code is in appendix) Here is how it works briefly.

(1) Photos are cut into small photos which only contain one gel in each of them.

(2) The square-shaped gel is found in each photo while the side length(L) is measured and averaged.

(3) ΔL/L at a particular time is calculated

(4) Plot ΔL/L with time (in hours)

The standard deviation is also calculated at each time point and plotted together with ΔL/L. For Am-5Bis gel, sometimes the gels were too dim to find with the MATLAB code described. If so, an extra step is required. The raw photos were treated by flat fielding to have a brighter view and a more significant contrast against the background. In this case, the remaining protocol is the same as the previously described process.

2.4.2 Process of reversing experiments

After the swelling experiments, all the solutions in the wells are drained with pipettes and replaced by 100μL 1×TAE/12.5×10^{-3} M Mg^{2+} buffer while the gels are left inside the wells. The buffer that was added is then drained with pipettes after at least 30 minutes' soaking and replaced by 150μL 60μM reversal solution. The position of the gels needs to be quickly adjusted to let the gels lay flat in the center on the bottom of the wells, followed by the above photo-taking process. Photos are taken every 30 minutes for 25h and processed according to the above protocol.

2.5 Measurements of hairpin intake in hydrogel during swelling

During the swelling experiments, hairpin intake after a time duration of swelling is measured by removing the hairpin solution after the prescribed time from the hydrogel undergoing swelling. The solution is moved to a 600μL tube. The volume of the removed hairpin solution is measuring using a pipette. (Pipettes with a maximum of 200uL are used in this experiment). The solution removed from the hydrogels is diluted 100 times with 1×TAE/12.5×10^{-3} M Mg^{2+} to test the solution's absorbance at 260nm with a biophotometer. The total amount of hairpin in the solution is then calculated based on the absorbance,total volume, as well as the standard curve (Figure 6). The gel's intake is then measured by subtracting amount of hairpin in the remaining solution from the total amount of hairpins originally adding in solution to a well containing a hydrogel.

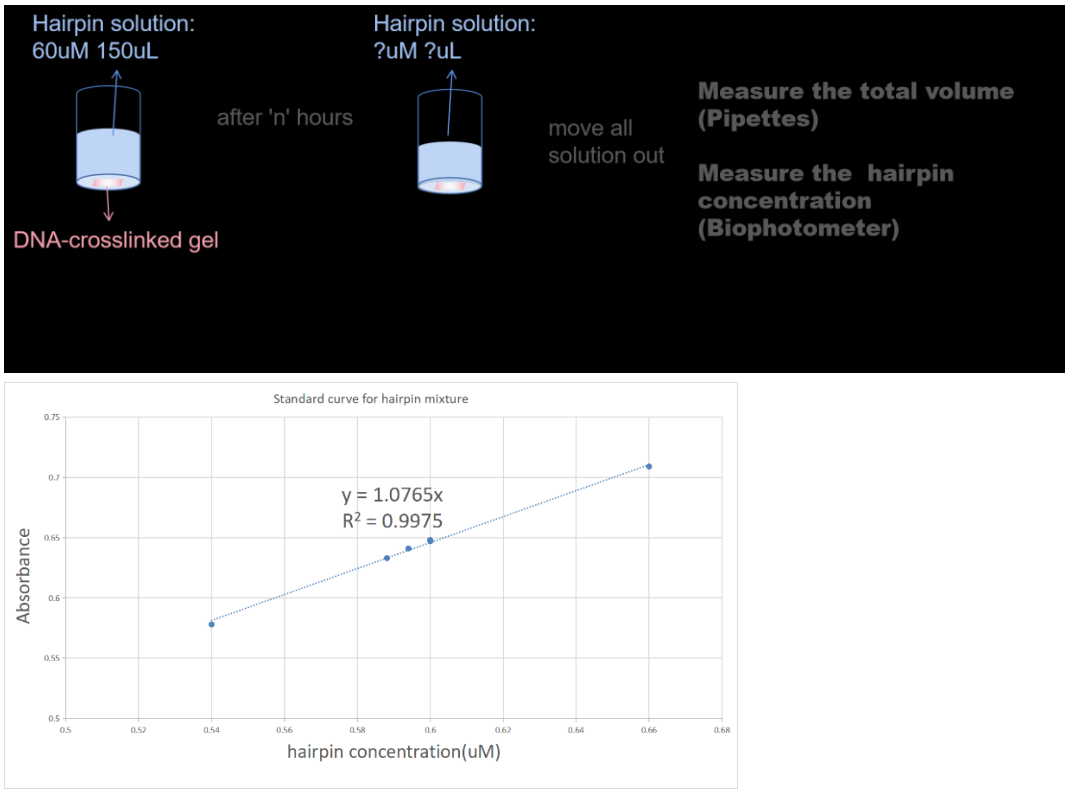

Figure 6 Process of mass balance experiments

3 Results and discussion

3.1 Swelling and reversing experiments for four different DNA systems

3.1.1 Swelling and reversing results of PEGDA10k hydrogel

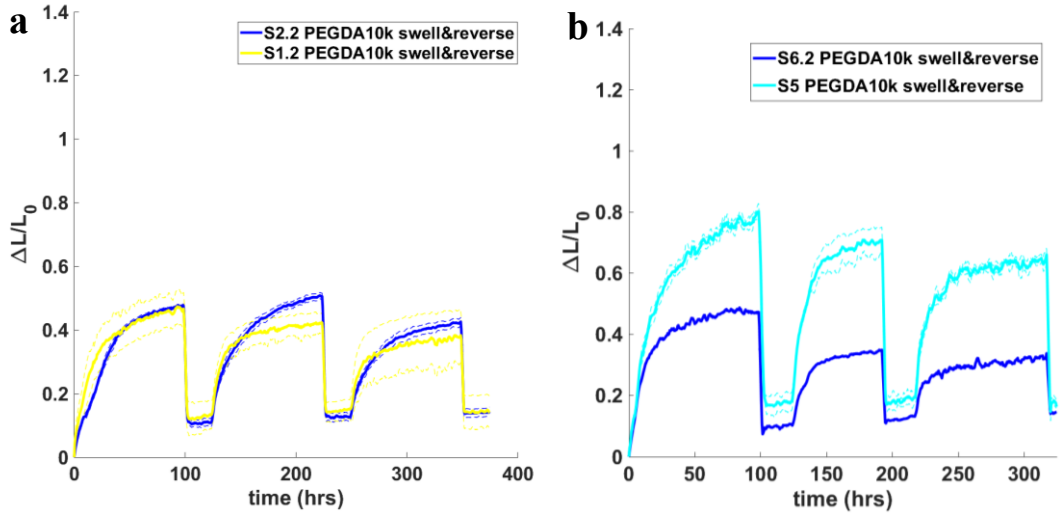

Figure 7 3 rounds of swelling and reversing of PEGDA10k hydrogels. Swell and reverse with 150μL 60μM hairpin solution (contain 1% terminator). a) S2.2 (N=4) & S1.2 (N=4) PEGDA10k gel. b) S5 (N=2) & S6.2 (N=1) PEGDA10k gel. Dash lines show the standard deviation of the data.

Figure 7 shows the changes in hydrogel length during swelling and reversal cycles for 4 different DNA systems.

Comparatively, System 5 swelled most. The amount of swelling slightly decreased as the swelling and reversing reached the second-round experiment. Then the swelling generally seemed constant in the following round of experiments. At the same time, reversing results did not change very much. Swelling decreased up to 23% in System 6.2 in the second round, but it was still a significant swelling compared to the original gel. This phenomenon might come from a slight polymer structure transition after the hairpins were knocked from the DNA crosslinks. When all the hairpins combined with reversals and diffused out of gel, the broken

DNA crosslink would tend to hybridize with its complementary strand. However, during this process, the DNA crosslinks might fail to hybridize (Figure 8). DNA-crosslinks make the two polymer chains closer to each other, which forms a repulsion between polymer chains. The repulsion will not vanish until the crosslinks broken. That is where the tension comes from. The gels would spontaneously reach a state with lower tension, the structure of which had a slight difference with the original gels. To reach that lower tense state, probably not all crosslinks were hybridized. It was the state with relatively lower energy, other than the original state, that was reached at the end of every reversing experiment. That could be the reason why the gel was unable to go back to its original state after reversing experiments. Because the polymer structure at the beginning of the second round of swelling was different from that at the beginning of the first round of swelling but was the same as that at the beginning of the third round of swelling, there was a slight difference between the first round of swelling and the second round of swelling but almost no difference between the second and third round.

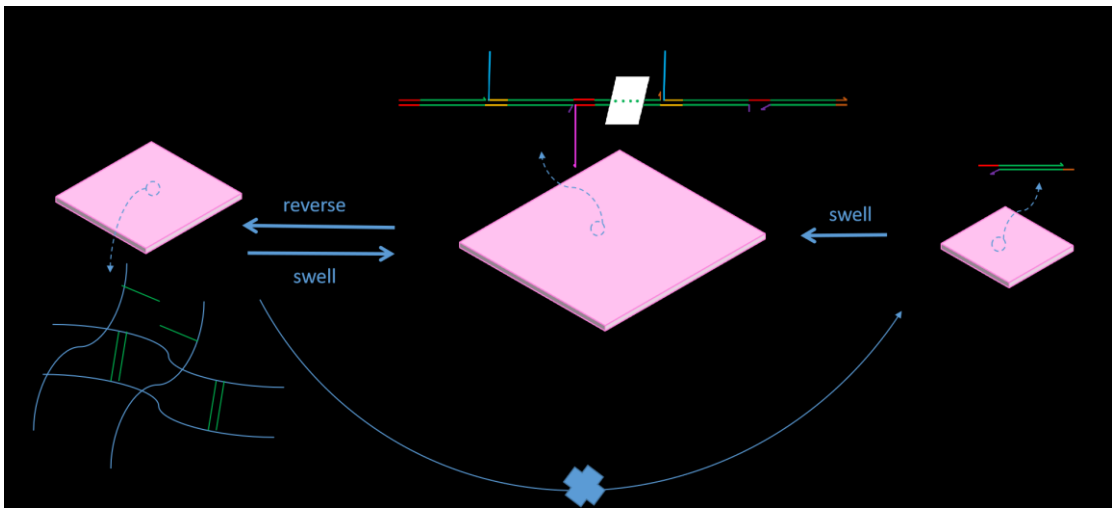

Figure 8 Difference between the original state and the state after reversing

3.1.2 Swelling and reversing results of Am-5Bis hydrogel

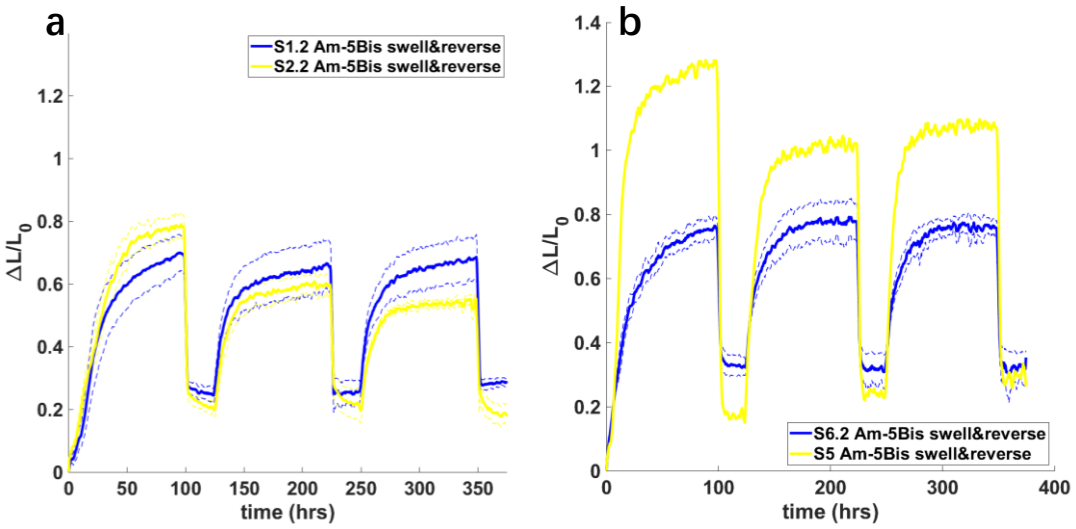

Figure 9 3 rounds of swelling and reversing of Am-5Bis hydrogel. Swell and reverse with 150μL 60μM hairpin solution (contain 1% terminator). a) S1.2 (N=3) & S2.2 (N=4) Am-5Bis hydrogel. b) S5 (N=1) &S 6.2 (N=4) Am-5Bis hydrogel. Dash lines show the standard deviation of the data.

Compared with PEGDA10k gels, Am-5Bis gels had a higher degree of swelling at equilibrium in all four systems. (Figure 9) We tried to qualitatively explain this swelling difference between different gels with the help of the Maxwell model in Thermodynamics[35-38]. The force between two crosslinked polymer chains can be simplified to a dashpot part and a spring part. The insertion of hairpin overcame the intermolecular forces between polymer chains and pushed the two polymer chains apart. This behavior led to increased internal tension. When the crosslink became longer, the elasticity entropy[37] of the polymer chains decreased (Elastic entropy only related to the number of states. Whether it is Am or PEGDA10k did not matter in terms of entropy), and the internal tension increased. The total increased energy was compensated by the hybridization energy of the insertion of the hairpin. When the insertion of hairpin into the crosslink could not compensate for the increase of total energy in polymer, gels

would no longer swell. Since PEGDA10k gel was more elastic than Am-5Bis gel[32-33], resistance increased faster in PEGDA10k than in Am-5Bis when crosslinks elongated, which indicated that less hairpin would be inserted into crosslinks in PEGDA10k gel. Less hairpin inserted resulted in a smaller swelling result.

System 6.2 and System 1.2 Am-5Bis showed a very constant swelling and reversing result during the three rounds of experiments, which meant that they had great potential to be a stable device in soft robots that could respond to DNA hairpin signals.

If the swelling and reversing results of two gels were put together, we could easily find common ground in all systems of two gels. The reversing would finish in 3 hours, while the swelling process would take 80h to finish. Part of the reason is that swelling was an entropy-unfavored and enthalpy-favored process while reversing was both entropy-favored and enthalpy-favored. When it comes to using the DNA-crosslinked hydrogel in soft robots, it would be better once the swelling could be faster. If we would like to accelerate the swelling of the hydrogel, it was necessary to have a deeper understanding of what happened in the hydrogels during swelling and what factors influenced that.

So, the mass balance experiments were designed to investigate the relationship between hairpin intake and swelling.

3.2 Mass balance in S5 Am-5Bis gel during swelling

The mass balance experiments were performed according to Chapter 2.5 in this thesis. System 5 hydrogels swelled most among all the systems, so System 5 was selected to perform the mass balance experiments.

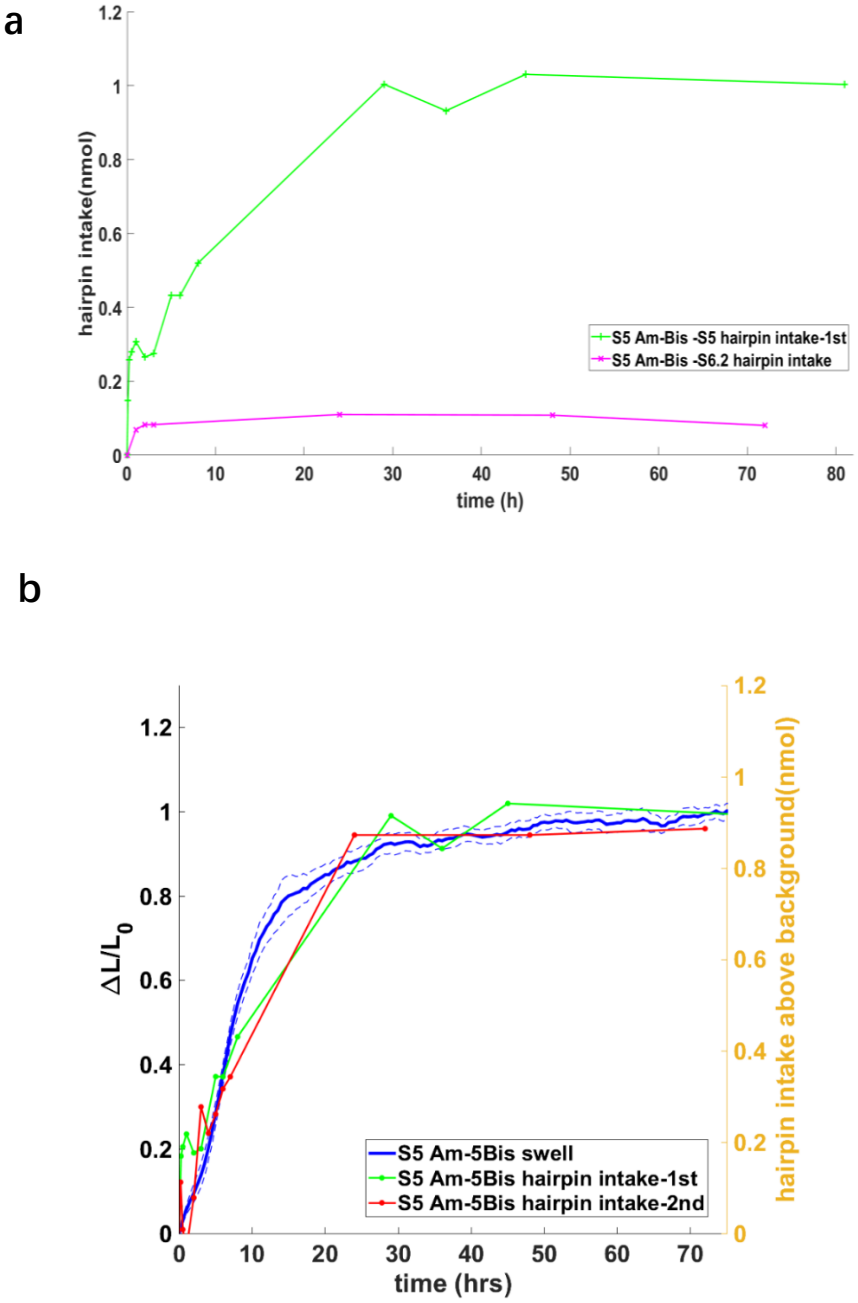

Figure 10 Hairpin intake of S5 Am-5Bis gel. Swell with 150μL 60μM hairpin solution. a) Hairpin intake for S5 Am-5Bis gel when swelling with S5 hairpin (contain 1% terminator) and S6.2 hairpin (wrong hairpin). b) Swelling (N=4) and two parallel mass balance experiments. Dash lines show the standard deviation of the data.

In Figure10(a), System 6.2 hairpins and System 5 hairpins were used to swell the System 5 Am-5Bis hydrogel, respectively. Though wrong hairpins would never swell the gel, they could

be taken up by the hydrogel, either because of adsorption or due to diffusion into the gel[34]. These hairpins formed the background of hairpin intake of the Am-5Bis hydrogels. So, in the following experiments related to Am-5Bis gels, this hairpin intake background was removed from the whole hairpin intake amount.

We performed the same experiments, swelling System 5 Am-5Bis gel with System 5 hairpins two times to test whether the mass balance experiment was repeatable. Figure10(b) showed that the two parallel mass balance experiments had almost the same result. Furthermore, the hairpin intake was comparatively consistent with the swelling process in Am-5Bis gels. If we assumed that all the hairpins that were taken up by the Am-5Bis gel were inserted into the crosslink, the number of hairpins inserted in each crosslink could be calculated easily.

To check how some factors affected the swelling and hairpin intake, we did some parameter investigation on System 5 Am-5Bis gels.

3.2.1 Influence of DNA crosslink concentration in Am-5Bis gels

In the next experiment (Figure 11), we measured the amount of hairpin uptake during swelling for hydrogels with different DNA crosslink concentrations. In the first five hours of these experiments there was some variance in the amount of measured hairpin intake in the first few hours. We believe the most reliable data in this experiment was captured between 10h and 80h.

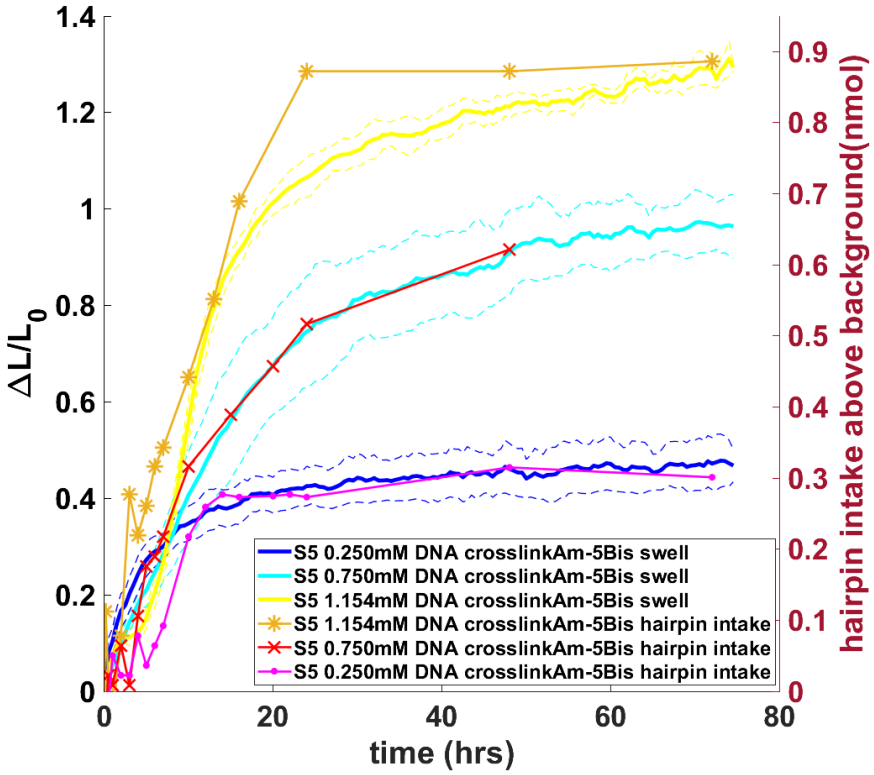

Figure 11 Swelling and hairpin intake of Am-5Bis hydrogel with 1.154mM crosslink (N=3), 0.75mM crosslink(N=2) and 0.25mM crosslink(N=3). Swell with 150μL 60μM S5 hairpin solution (contain 1% terminator). Dash lines show the standard deviation of the data.

Figure 11 illustrated that for all of the DNA crosslink concentrations tested, the speed of hairpin intake was always consistent with that of the swelling. One important conclusion from Figure 11 was that swelling and hairpin intake shared the speed when the concentration of the DNA hairpin solution was 60μM. We could also find that gel with a higher DNA crosslink concentration would have a more significant swelling result and uptake more DNA hairpins. However, the final swelling result seemed not to be proportional to DNA crosslink concentration. With the help of this equation,

$$Number \text{ of hairpin inserted} = \frac{\text{hairpin intake} \times 2}{Volume \text{ of gel} \times \text{ crosslink concentration}},$$

we could compare the number of DNA hairpins inserted into one DNA crosslink in one gel.

(Table 4).

Crosslink concentration (mM)	Crosslink/gel (nmol)	Hairpin intake at 72h (nmol)	Hairpins/crosslink (nmol)
1.154	0.185	0.89	10
0.75	0.120	0.65	11
0.25	0.040	0.30	15

Table 4 The number of hairpins that are inserted into each crosslink in the hydrogels

More hairpins were inserted into one crosslink in the gel with lower DNA crosslink concentration than were inserted into each crosslink on average in gels with higher DNA crosslink concentration.

It could also be explained by the Maxwell model[35-38]. Lower DNA crosslink concentration resulted in a lower force between two polymer chains, making the polymer chains easier to be pulled apart[36]. A lower resistance indicated that more DNA hairpins could be inserted into one DNA crosslink.

3.2.2 Influence of DNA hairpin concentration in Am-5Bis gels

We performed two experiments in which we measured how the amount of hairpin uptake was affected by the initial hairpin solution concentration (Figure 12):

(1) We used hairpin solutions of three different concentration (60uM, 40uM and 20uM) to swell the gel while keeping the total volume constant;(Figure 12a)

(2) We used hairpin solutions of three different concentration (60uM, 40uM and 20uM) to swell the gel while keeping the total hairpin amount (moles) constant. (Figure 12b)

From the pink bottom line in Figure 12a and the bottom two lines, light blue and yellow ones,

in Figure 12b, it was observed that the Am-5Bis in 20μM hairpin solutions did not swell until after ten hours. To explain it, raw photos during this experiment were posted below. It is always observed that the gel swells very strangely and not uniformly when it is swelling with a hairpin solution with a low concentration of hairpins.

In the first 6h in Figure 12a, we found that the hairpin intake slope was proportional to the hairpin concentration. That might indicate that diffusion controlled the swelling and hairpin intake. But after that, slopes of both swelling and hairpin intake continued decreasing. It was worth mentioning that, after 6h, swelling became quite slow while hairpin intake was still processing. That was a different behavior from a diffusion-controlled process. There should be a mechanism transition at or around that point.

After several hours, the gel went back to a square shape and swelled typically.

If we compared the slopes of hairpin intake with the slope of swelling results in the light blue line and the orange line (Figure 12b) between 10h to 50h (i.e. where the total volume of solution was varied but the hairpin concentration was kept constant), we would find that they are of the same speed. The blue and green lines, the other case where the volume is varied but the concentration remained the same, are also overlaid.

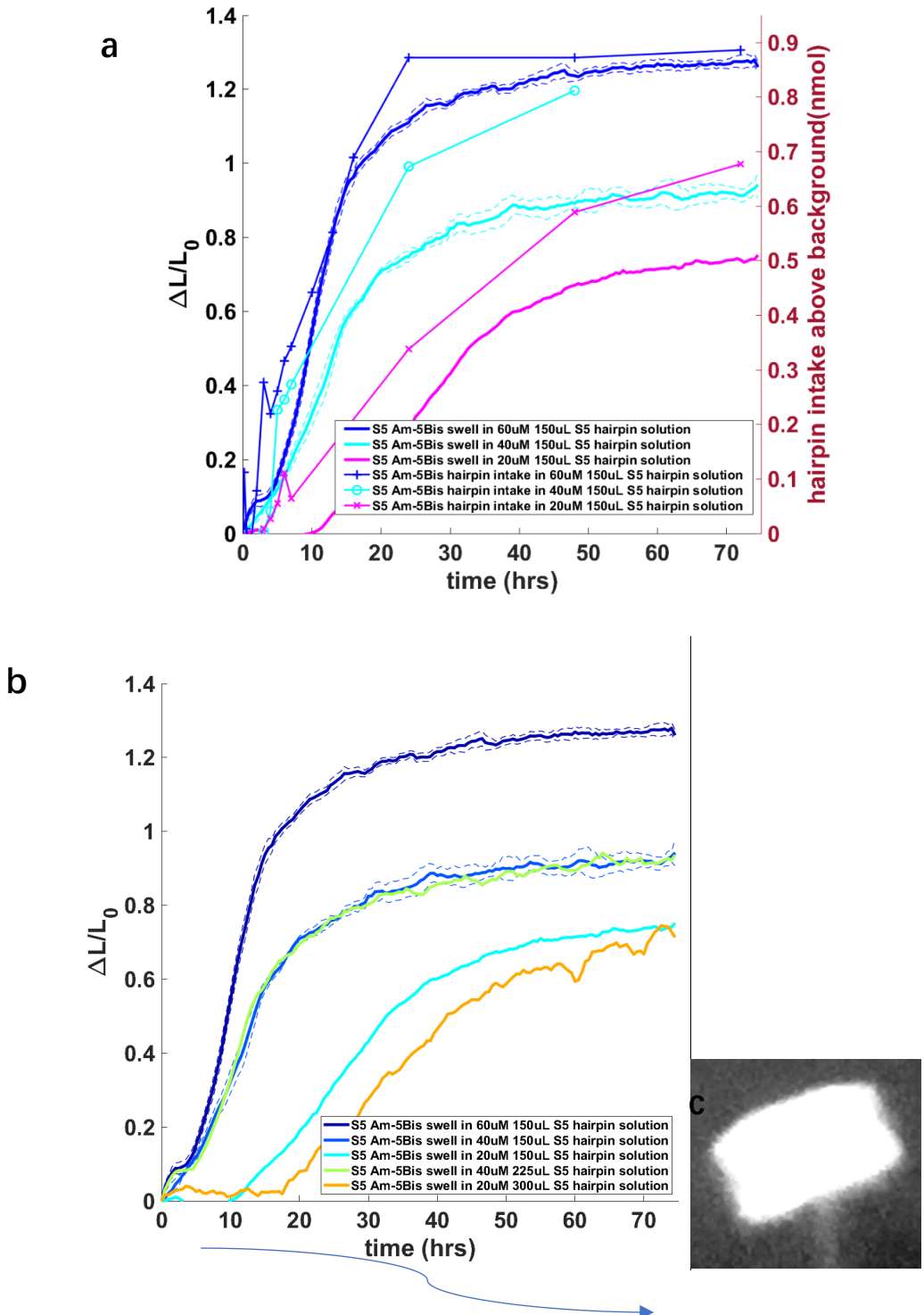

Figure 12 Swelling and hairpin intake of S5 Am-5Bis gel with different hairpin concentrations. a) Swelling and hairpin intake of S5 Am-5Bis gel when swelling with 60 (N=4), 40 (N=2), 20 (N=1) μM hairpin solution. b) Swelling of Am-5Bis gel with a different total amount of S5 hairpin (N=1 for the bottom two lines). c) One gel swelling at 10h. Dash lines show the standard deviation of the data.

The results suggest that number of hairpins was in excess during swelling, so that concentration

rather than the total number of moles was the parameter that determined the final amount of swelling. Neither the amount of swelling nor the amount of hairpin intake were proportional to the hairpin concentration.

Here we provided a hypothesis. We assume that hairpins can only insert into the crosslinks in the hydrogel when the resistance is overcome. In the Maxwell model, we pointed out that the resistance of hairpin insertion (decrease of polymer chain entropy and increase of internal tension[21,38]) grew more prominent with more hairpins inserted. In Diagram 1, ΔG should be more negative to make the reaction happen if the gradually strengthening resistance should be overcome. So, based on the Van 't Hoff isotherm, there should be a higher hairpin concentration inside the gel ($c_{hairpin}$) to enable the hairpin insertion reaction to happen. A more immense osmotic pressure can lift the hairpin concentration inside the gels, which is essential for the further insertion to take place when the swelling proceeds[39-41].

Diagram 1 Energy balance of the hairpin insertion reaction.

In the 60μM hairpin solution, the maximum hairpin concentration inside the gels was undoubtedly higher than in 40μM and 20μM solution. So, more insertion reactions would take place and the gels in that solution swelled most.

Based on this hypothesis and Figure 12, we could have two predictions:

(1) The equilibrium size and swelling rate of the hydrogel will increase in a solution of higher concentration.

(2) In the first several hours, the resistance is not so large, and it is easy to insert a hairpin. The transport of hairpins into the gel controls hairpin intake rate and swelling rate. As the hairpin insertion goes, the resistance becomes quite sizeable, which significantly slows down the hairpin insertion. Then the hairpin insertion reaction controls the hairpin intake rate and swelling rate. Diffusion of hairpins goes while the consumption of hairpins inside the gels slows down. That would result in hairpin accumulating inside the gels. The accumulated hairpins in the gel alleviates the charge imbalance between the inside and outside the gel, which hinders the hairpin transport and deaccelerates it. The accumulated hairpins inside the gel and the slow insertion reaction explain why hairpin intake goes while swelling almost stops in Figure 12a. When no charge imbalance exists and the hairpin concentration in the gels at that time is not high enough to overcome the resistance to insert one more hairpin, swelling ends. At that point, the hybridization energy of a hairpin insertion could not compensate for the too-large resistance.

3.3 Mass balance in System 5 PEGDA10k gel during swelling

Like System 5 Am-5Bis gels experiments, the background rate of hairpin uptake by hydrogels in the absence of Watson-Crick complementarity between the hairpins and the DNA in the crosslinks was measured for PEGDA10k gels. The results are presented in Figure 13a. This background was removed in the hairpin intake for the following PEGDA10k gels experiments.

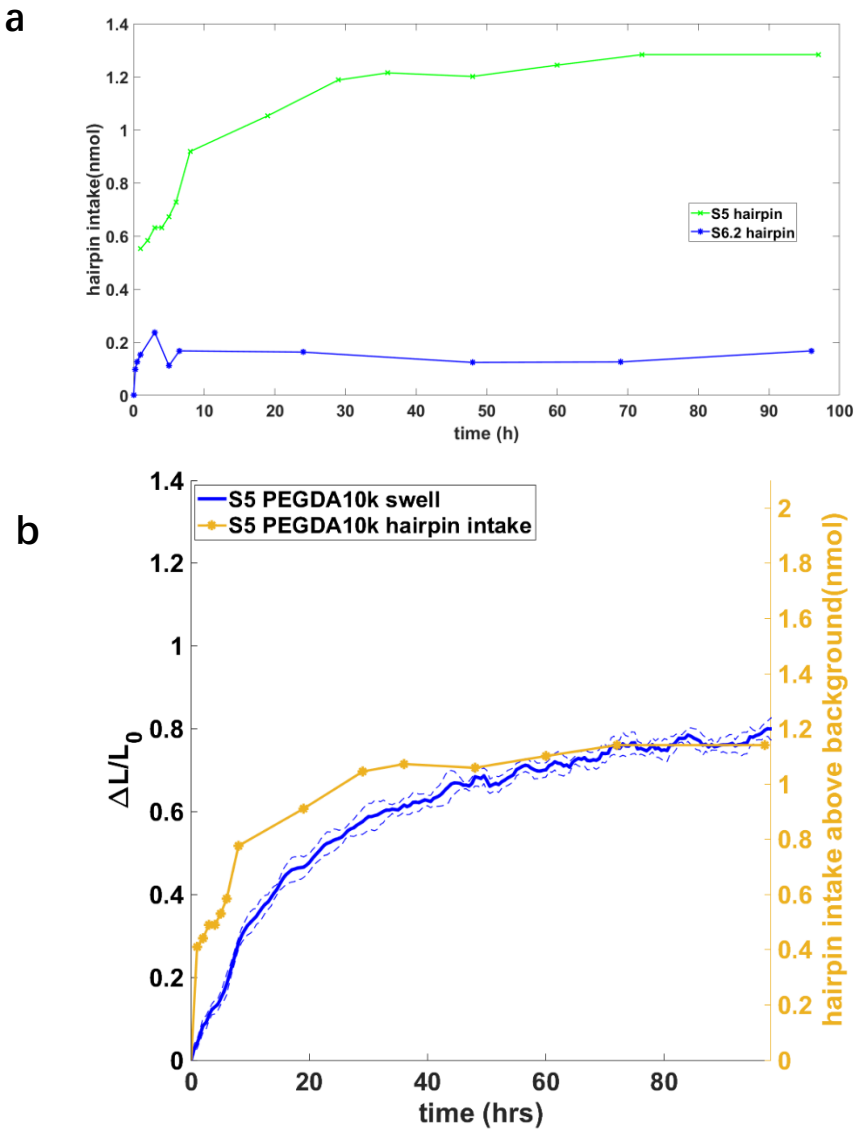

Figure 13 Swelling and hairpin intake of S5 PEGDA10k gel. Swelling took place in 150µL of a 60µM hairpin solution (contain 1% terminator). a) hairpin intake when swelling with S5 hairpin and S6.2 hairpin. b) Swelling (N=4) and hairpin intake of S5 PEGDA10k gel. Dash lines show the standard deviation of the data.

In Figure 13b, at 29h, the PEGDA10k gel took up 94% of the hairpins that it would take up after 100h, but the swelling result only reached 73% of the ΔL/L it would reach at 100h. There was thus a significant time lag between hairpin intake and swelling. The slope of the hairpin intake curve was also slightly larger than the slope of the swelling curve in the first 8h. Furthermore, while the swelling had just begun in the first hour, the hairpin intake had already reached 25% of the total hairpin intake. To learn more about what happened, we performed another experiment in which we measured the amount of hairpin uptake to more precisely measure how the amount of hairpin intake that occurred in the first hour. (Figure 14)

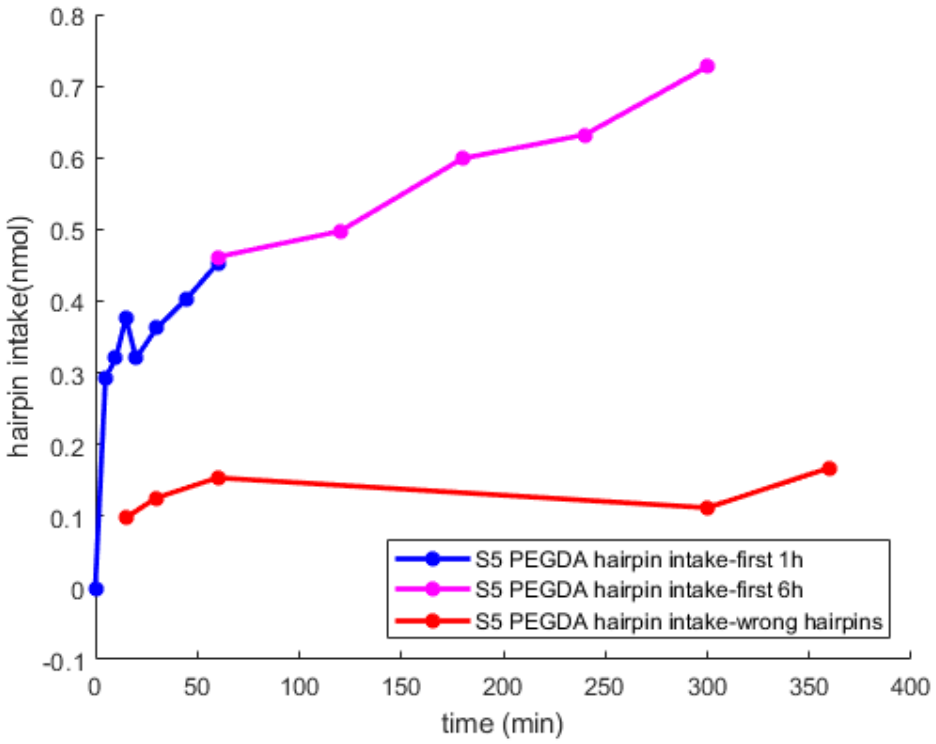

Figure 14 Hairpin intake of S5 PEGDA10k gel in the first 1h. Swelling occurred in 150μL of a 60μM S5 hairpin solution. (contain 1% terminator)

In the first hour after an initial transient, the hairpin intake was linear and the slope in the first hour was the same as the slope measured over the first 6 hours. One potential explanation for

the very rapid increase in the first time point is the sizeable osmotic pressure at the very beginning. At the beginning, no hairpins existed in the gels while the outside solution had 60μM hairpins1 and 60μM hairpin2. Since DNA hairpins were negatively charged, the imbalance of charge between inside and outside the gels created a sizeable osmotic pressure. Sizable osmotic pressure greatly accelerated the hairpins transport. At the same time, PEGDA10k gels would adsorb hairpins due to its porous structure and large surface area. The adsorption is a very rapid physical process. Many hairpins were adsorbed into the gels and mitigated the charge imbalance. So, after several minutes, the hairpin intake speed decreased.

Based on Figure 13b, at 29h, it seemed the hairpin intake nearly came to an end as the swelling process was progressing. One easy and straightforward experiment design for investigating what happened at that period was to replace the hairpin solution with a solution containing no hairpins at the time where we observed that hairpin intake was almost finished, but the swelling process was only halfway complete. An experiment of this design is diagramed in Figure 15.

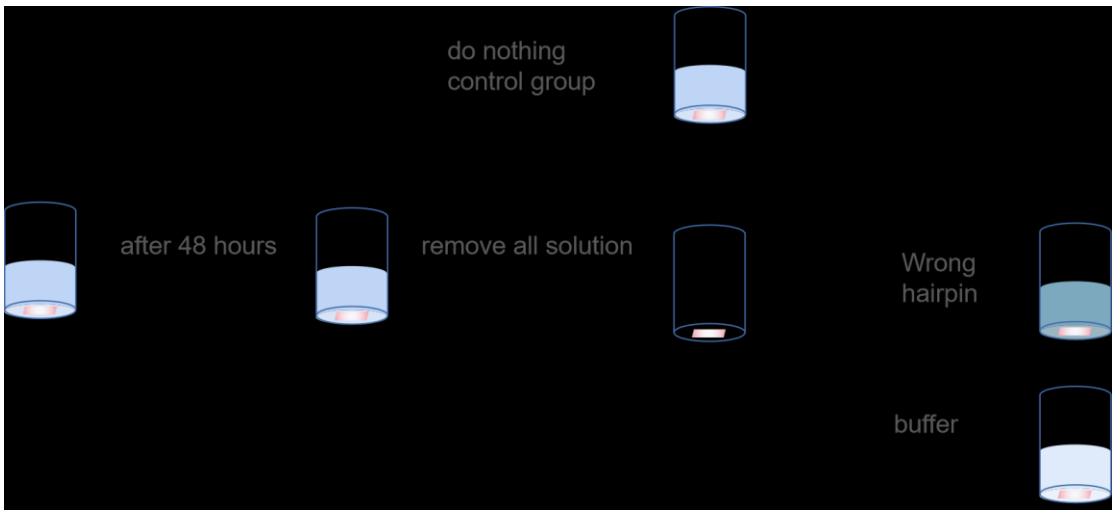

Figure 15 Experiment design of changing hairpin solution when hairpin intake did not vary too much with time

To ensure that the hairpin intake is almost finished, we picked the '49.5h' as the time point to

replace the buffer. (Figure16)

According to the above assumption, if the hairpins in solution after 49.5h played no role, changed the solution surrounding the hydrogel to a solution that contained the S6.2 hairpin (wrong hairpin) would not affect the swelling process. However, we saw in Figure 16b that after changing the buffer, the gel with the wrong hairpins swelled a bit and then did not change while the gel with the right hairpins continued to swell. Most interestingly, when we changed the System 5 hairpin solution to pure 1×TAE/Mg^{2+} buffer, the side length of the gel decreased 10%. This finding strongly disagreed with the hypothesis that hairpin intake in PEGDA10k was exactly the hairpin inserted.

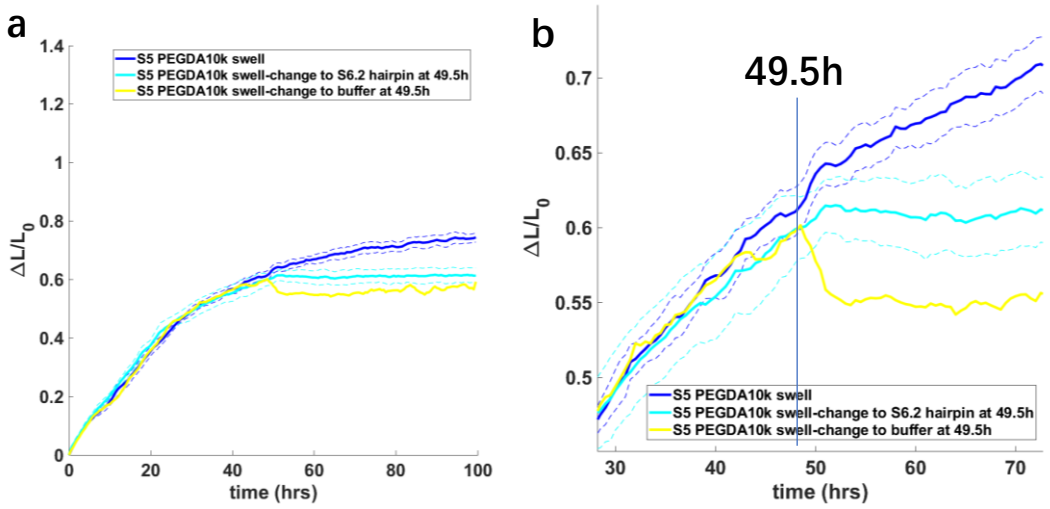

Figure 16 a) Swelling of PEGDA10k gel with 150μL 60μM hairpin solution (contain 1% terminator) for 49.5h. Then swelling with buffer (N=1)and 51μM wrong hairpin solutions (N=3) for the rest of time. The control group (blue line) had three gels. (N=3) b) Swelling detail at t=49.5h. Dash lines show the standard deviation of the data.

To explain the phenomenon in Figure 16b, one option is to consider the threshold hypothesis presented in Section 3.2.2 in this thesis, the threshold hypothesis. The reason for a slight swelling in the wrong hairpin buffer would be that System 5 hairpins trapped inside the gel

that was not washed away had the tendency to insert into the crosslink. The presence of the wrong hairpin, System 6.2 hairpin, provided the osmotic pressure to facilitate the insertion, even if it could not insert on its own. When the slight amount of System 5 hairpin inside the gel inserted into the crosslink, the swelling ended even though the osmotic pressure is enough. (Or, in other words, the hairpin concentration was above the threshold, but it was the wrong hairpins).

For the gel in the pure buffer, when the System 5 hairpin solution was removed and replaced by a pure buffer, the osmotic pressure outside the gel became extremely small. To balance the charge[35] again, some DNA hairpins may diffuse out, and the hydrogel shrunk accordingly. At that time, the insertion reaction would happen in the inversion direction, which resulted in the dissociation of hairpin from the crosslink. The dissociation and diffusion shortened the DNA crosslink, which caused the gel to shrink. When the osmotic pressure was balanced again or when the osmotic pressure was not enough to overcome the energy barrier of dissociating inserted hairpins, dissociation stopped, and the gel reached its final size.

Here is a summary of our findings and hypothesis of the swelling process. We propose that the driving force for the insertion of the hairpin was the hybridization energy of forming more hydrogen bonds between nucleobases. The resistance was composed of 1) decrease of elasticity entropy and 2) increase of internal tension. At the beginning of swelling, the resistance was small, so the insertion was relatively rapid. However, as more hairpins were inserted, the hairpin insertion slowed down and hairpin accumulated inside the gels. The accumulation of hairpins gradually decreased the osmotic pressure and deaccelerate the

diffusion, while resistance continued to increase. Finally, when the driving force was more minor than resistance, hairpin insertion ended, and the gel stopped swelling.

4 Conclusion

In this thesis we investigated DNA-controlled reversible swelling hydrogel system and observed the high-degree swelling and reversing of PEGDA10k and Am-5Bis hydrogels. Four different systems of DNA crosslinks and DNA hairpins that were orthogonal to each other, designed by Kuan-Lin Chen, were considered. We also developed experiments designed to characterize the amount of hairpin uptake by hydrogels over time during swelling, which made it possible to relate the degree of a gel's swelling and its hairpin intake. Moreover, we considered, qualitatively a Maxwell model to evaluate whether it could explain some of the phenomena we observed during the experiments.

In conclusion, we have the following findings:

1) DNA hydrogel with higher DNA crosslink concentration would have a more significant equilibrium swelling result.

2) The gel swelled with a hairpin solution of higher concentration showed a more significant swelling result and a faster swelling rate.

3) An insertion reaction can happen inversely when the osmotic pressure was in the opposite direction. The phenomena that hairpin intake and swelling results are not proportional to the hairpin solution concentration also demonstrated the vital role of osmotic pressure.

4) The reason why PEGDA10k gel swelled less than Am-5Bis gel with the same system of

DNA could be that the intermolecular forces between PEGDA10k polymer chains were higher than Am-5Bis gel. In that situation, the resistance (composed of the tension of polymer chain when pulled apart and elastic entropy) for the insertion of one hairpin was higher in PEGDA10k gel, finally resulting in a smaller number of hairpins inserted into the crosslink in PEGDA10k gels.

5) There are three stages during this DNA sequence-directed swelling.

Stage 1: In the first several minutes, the rapid adsorption and diffusion induced by sizeable osmotic pressure make the hairpin intake rapidly increase. Osmotic pressure decreases a lot because many hairpins enter the gel and alleviate the charge imbalance. No hairpin insertion happens, so no swelling is observed there.

Stage 2: The two rapid physical processes finish. The resistance of hairpin insertion is small at the beginning of swelling (in the first 6h), so any hairpins diffusing into the gel will be inserted into the crosslinks. Diffusion rate, which is proportional to the hairpin solution concentration, controls the swelling and hairpin intake. So, the swelling rate (slope of swelling curve) and hairpin intake rate (slope of hairpin intake curve) are proportional to the solution concentration.

Stage 3: After swelling for 6h, the resistance becomes stronger and slows down the hairpin insertion reactions. The swelling becomes very slow. Due to the continuous diffusion and slowing hairpin insertion reactions, hairpins accumulate in the gels, which alleviates the charge imbalance and deaccelerates the diffusion. (The slope of hairpin intake decreases, but the hairpins are still entering the gels.) When osmotic pressure is balanced between inside and outside the hydrogels, diffusion reaches the equilibrium. From then on, hairpin intake doesn’t change any more. That also explains why hairpin intake is still increasing while swelling

doesn’t vary too much. (light blue lines and pink lines in Figure 12a.)

These findings and insights may help us better understand the reversible high-degree swelling hydrogel and provide some new ideas to improve it and utilize it.

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Appendices

DNA sequences

a. System 5:

A: /5Acryd/GGT GTA AGG TGA GGG TGA TGG TAA

R: /5Acryd/CTA TCT ATC CAT CAC CCT CAC CTT AC

Hairpin1: TTA CCA TCA CCC TCA CCT TAC TTG TAG ATT TTT TGT AAG GTG AGG GTG ATG GAT AGA TAG GGT AGG TGA ATG GGA

Hairpin2: TAT GAG TGA GTT AGG ATC TAC AAG TAA GGT GAG GGT GAT GGT TTT TCT ATC TAT CCA TCA CCC TCA CCT TAC ACC

Terminator hairpin1: TTA CCA TCA CCC TCA CCT TAC CTC TCC ACT TTT TGT AAG GTG AGG GTG ATG GAT AGA TAG GGT AGG TGA ATG GGA

Terminator hairpin2: TAT GAG TGA GTT AGG ATC TAC AAG TAA GGT GAG GGT GAT GGT TTT TAC CAG CCT CCA TCA CCC TCA CCT TAC ACC

Reversal1: TCC CAT TCA CCT ACC ATA GAT AGC CAT CAC CCT CAC CTT AC

Reversal2: CCA TCA CCC TCA CCT TAC TTG TAG ATC CTA ACT CAC TCA TA

b. System 6.2:

A: /5Acryd/CTA CCA CTC CAC TCA CAC TCC ACT CC

R: /5Acryd/GGT GGA GTG GAG TGT GAG TGG GAT

Hairpin1: ATC CCA CTC ACA CTC CAC TCC CGC TCG CCT AAT AGG AGT GGA GTG TGA GTG GAG

TGG TAG GTT TAG GTG AGG TGG

Hairpin2: TTG TAA GTG AGA GTG GCG AGC GGG AGT GGA GTG TGA GTG GTA ATA CTA CCA CTC CAC TCA CAC TCC ACT CCA CC

Terminator hairpin1: ATC CCA CTC ACA CTC CAC TCC GTG CTG GTT AAT AGG AGT GGA GTG TGA GTG GAG TGG TAG GTT TAG GTG AGG TGG

Terminator hairpin2: GTT GTA AGT GAG AGT GGC GAG CGG GAG TGG AGT GTG AGT GGT AAT AAA GGC GTC CCA CTC ACA CTC CAC TCC ACC

Reversal1: CCA CCT CAC CTA AAC CTA CCA CTC CAC TCA CAC TCC ACT CC

Reversal2: CCA CTC ACA CTC CAC TCC CGC TCG CCA CTC TCA CTT ACA AC

c. System 1.2:

A: /5Acryd/CCT AAG TTC GCT GTG GCA CCT GCA CG

R: /5Acryd/CAA CGT GCA GGT GCC ACA GCG TGG

Hairpin1: CCA CGC TGT GGC ACC TGC ACG CAC CCA CAG CCA TCG TGC AGG TGC CAC AGC GAA CTT AGG ATG ATT GTG TAT AGT

Hairpin2: AGT TAA GAG AAT GAT TGT GGG TGC GTG CAG GTG CCA CAG CGG CCA TCC TAA GTT CGC TGT GGC ACC TGC ACG TTG

Terminator hairpin1: CCA CGC TGT GGC ACC TGC ACG TAG ACT TTT TTC GTG CAG GTG CCA CAG CGA ACT TAA TGA TTG TGT ATA GT

Terminator hairpin2: AGT TAA GAG AAT GAT TGG GTG CGT GCA GGT GCC ACA GCG TTT TTG CGT AGC GCT GTG GCA CCT GCA CGT TG

Reversal1: ACT ATA CAC AAT CAT CCT AAG TTC GCT GTG GCA CCT GCA CG

Reversal2: CGC TGT GGC ACC TGC ACG CAC CCA CAA TCA TTC TCT TAA CT

d. System 2.2:

A: /5Acryd/CTC TGT CTG CCT ACC ACT CCG TTG CG

R: /5Acryd/ATT CGC AAC GGA GTG GTA GGC TTT

Hairpin1: AAA GCC TAC CAC TCC GTT GCG GAA CCT CC TAA ACG CAA CGG AGT GGT AGG CAG ACA GAG GTA AGG TAA GAT AGG

Hairpin2: GGG TAG TGT GAT GTG GGA GGT TCC GCA ACG GAG TGG TAG GCC TAA ACT CTG TCT GCC TAC CAC TCC GTT GCG AAT

Terminator hairpin1: CCA CGC TGT GGC ACC TGC ACG TAG ACT TTT TTC GTG CAG GTG CCA CAG CGA ACT TAA TGA TTG TGT ATA GT

Terminator hairpin2: AGT TAA GAG AAT GAT TGG GTG CGT GCA GGT GCC ACA GCG TTT TTG CGT AGC GCT GTG GCA CCT GCA CGT TG

Reversal1: CCT ATC TTA CCT TAC CTC TGT CTG CCT ACC ACT CCG TTG CG

Reversal2: GCC TAC CAC TCC GTT GCG GAA CCT CCC ACA TCA CAC TAC CC

Appendices

Matlab code

i.To cut the picture

```
% function GelImager_CropAndSortBeads_TSeries()
% Calculate the degree of swelling based upon the area calculated
from the
% fluorescence data. Fluorescence of a bead gives a 2D projection of
the
% "thickest" part of the bead, providing a semi-reasonable estimate
of the
% total size of the bead. Calculation is currently based upon a
single bead
% per image and the images progress through time. Saves a mat file
with the
% calculated data for cross-well plotting using a different function.

clear; close all;
% Important parameters:
ExportPlot = 0;
[Filename,Pathname]=uigetfile('*.tif', 'SELECT THE
GELS','C:\Users\jfernace\Desktop\Schulman Lab\Projects\DNA-
crosslinked Hydrogels\','MultiSelect','on');
addpath(Pathname);
NumBeads = 4;
MakeFolders = 1;
% IMPORTANT:!!!!!
InitBeadNum = 1;
%%
if iscell(Filename)
    num_images=numel(Filename);
else
    TempF{1} = Filename;
    Filename = TempF;
    num_images=1;
end
TIFdir=[Pathname 'PNGs'];
BeadMatDir = [Pathname 'BeadImgs'];
if MakeFolders == 1
    mkdir(TIFdir);
    mkdir(BeadMatDir);
end
```

```
InitImgNum = 1;
UberImg = imread([Pathname Filename{InitImgNum}]);
ffig=figure(1);
imshow(UberImg,[])
title(['Get MB Locs. Img Num:' num2str(InitImgNum)])
[Xpts, Ypts] = getpts(ffig);




for ImgNum = 1:num_images
    UberImg = imread([Pathname Filename{ImgNum}]);

    for BeadNum = 1:numel(Xpts)
        UsedBeadNum = InitBeadNum + BeadNum - 1;
        if MakeFolders == 1 && ImgNum == 1
            mkdir(BeadMatDir,['mb_' num2str(UsedBeadNum)]);
        end
        IMG_data = imcrop(UberImg,[Xpts(BeadNum)-150 Ypts(BeadNum)-150
300 300]);
        %% Save the Data:
        if MakeFolders == 1
            save([BeadMatDir '\mb_' num2str(UsedBeadNum) '\'
Filename{ImgNum}(1:end-4) '_mb' num2str(UsedBeadNum)
'.mat'],'IMG_data')
            IMG8bit =
histogram_stretch(IMG_data,min(min(IMG_data)),max(max(IMG_data))-
min(min(IMG_data))); %stretch the data to make it visible in 8 bit
format/png format (actually 0-1)
            imwrite(IMG8bit,[TIFdir '\' Filename{ImgNum}(1:end-4) '_mb'
num2str(UsedBeadNum) '.png'],'PNG'); %Write the intensities to a png
file
        else
            save([Pathname Filename{ImgNum}(1:end-4) '_mb'
num2str(UsedBeadNum) '.mat'],'IMG_data')
            IMG8bit =
histogram_stretch(IMG_data,min(min(IMG_data)),max(max(IMG_data))-
min(min(IMG_data))); %stretch the data to make it visible in 8 bit
format/png format (actually 0-1)
            imwrite(IMG8bit,[Pathname Filename{ImgNum}(1:end-4) '_mb'
num2str(UsedBeadNum) '.png'],'PNG'); %Write the intensities to a png
file
        end
    end
```

```
end
```

ii.To find the square, measure the side lengths and calculate ΔL/L for each square

```
% function GelImg_GlobThreshSquareSwell()
clear; close all;
%======================
% File Path:
%======================
BasePath = 'C:\Users\jfernace\Desktop\Schulman Lab\Projects\DNA-crosslinked Hydrogels\DNAGelRachel\normal, bright\mb_10\';

[Filename,Pathname]=uigetfile('*.mat', 'Select Data to Compare',BasePath,'MultiSelect','on');
addpath(Pathname);

if ~iscell(Filename)
    FilenameTemp{1} = Filename;
    Filename = FilenameTemp;
end
Filename = Filename';
% ReadOrder = [4 1 3];
% ReadOrder = [2 1 3:numel(Filename)];Filename = Filename(ReadOrder);

num_images=numel(Filename);
SpecMess = 'SLGlobTh';
%%
%======================
% Time Profile:
%======================
TimeDiff = 0.5;
TimeStamps = linspace(0,0.5*(numel(Filename)-1),numel(Filename)); % hrs
% TimeStamps = [0 1.167 linspace(1.5,(num_images-3)*TimeDiff,num_images-2)];
% TimeStamps = [-1 -0.5 linspace(0,(num_images-3)*TimeDiff,num_images-2)];
% TimeStamps = [-0.5 linspace(0,(num_images-2)*TimeDiff,num_images-1)];

%%
%======================
% Find Object Outline:
```

```
%======================
MaskedObjArea=[];
clear Archive
for ImgNum = 1:num_images
    RawDat=load(Filename{ImgNum});
    IMGData_Doub = imadjust(RawDat.IMG_data);

    %======================
    % Important parameters to be adjusted:
    % hsize: usually 55 is best. sometimes using values like 45, 75,
or 95 can help,
    % but often will make it worse. very situational

    % ThLinVar: user defined vector of numbers for adjusting the
threhsold.
    % Put two numbers in the field (can be same number).
    % Goal is to make as large as possible without adding excess
background to
    % the object. Chose to vary over the time series because
    % the gels get dimmer/higher background intensity. Numbers can
    % vary wildly. Sometimes need to go as low as "15" for second
number.
    % Higher numbers equal higher areas in the end.

    % RotateDegrees: if rotation will help (if extra things pointing
straight off side of
    % object that will disrupt the extrema analysis). Rotation in
degrees to counterclockwise
    hsize=85;%55 %35for am5bis %95 for pegda20k
    ThLinVar = [linspace(100,150,150)];
    RotateDegrees = 0;
    %======================

    sigma=110; %110
    FiltDat = IMGlpfilter(IMGData_Doub,hsize,sigma); % low pass
filtering of image
    if RotateDegrees ~= 0 % rotate if you need to
        FiltDatTh = imrotate(FiltDat,RotateDegrees);
        IMGData_Doub = imrotate(IMGData_Doub,RotateDegrees);
    else
        FiltDatTh = FiltDat;
    end
    ThFudgeFact = 1.35; %1.35
    MaskedIMG2 = FiltDatTh >= ThFudgeFact* mean(FiltDatTh(:)); %
```

```
thresholding (rnd 1)
    ThFudgeFact =  mean(FiltDatTh(MaskedIMG2))/
mean(FiltDatTh(~MaskedIMG2))/ThLinVar(ImgNum);
    ThFFSaved(ImgNum,1) = ThFudgeFact;
    MaskedIMG2 = FiltDatTh >= ThFudgeFact * mean(FiltDatTh(:)); %
thresholding (rnd 2)

    %=========
    % If you have small squares you might need to lower this number.
    % This removes all objects with an area less than this many
pixels
    MaskedIMG2=bwareaopen(MaskedIMG2,800);
    %=========

    MaskedIMG2Bounds=bwboundaries(MaskedIMG2,8);
    Masked_ConnComps = bwconncomp(MaskedIMG2,8);

MaskedObjArea{ImgNum,1}=regionprops(Masked_ConnComps,'Area','centroid
','extrema','PixelIdxList'); % Make array just in case it detects
multiple objects. Also this is why we use regionprops instead of
bwarea
    % Save the remaining data into an archive structure
    Archive.IMG(:,:,ImgNum) = IMGData_Doub;
    Archive.MaskedIMG{ImgNum} = MaskedIMG2;
    Archive.AllMaskedBounds{ImgNum,1} = MaskedIMG2Bounds;
    Archive.ConComps{ImgNum,1} = Masked_ConnComps;
end
%======================
% Get Hydrogel Areas, Boundaries, and Side Lengths:
%======================
% Extract the area and the object boundaries. If more than one object
was
% found from the mask, pick the one with a centroid in the middle of
the image.
% This works well unless the background object is a ring around the
center...
ObjAreaSeries=[];
ImgCenter = size(IMGData_Doub)/2;
isCurledIndicator = zeros(numel(Archive.ConComps),1);
ClustPtsDist=[];
ExtremPts=[];
for ImgNum = 1:num_images
    NumComponents = Archive.ConComps{ImgNum}.NumObjects;
    TempAreaVals = [];
```

```
    if NumComponents == 0
        ObjAreaSeries(ImgNum,:) = 0;
        Archive.MaskedBounds{ImgNum,1} = NaN;
        ExtremPts{ImgNum} = [NaN NaN];
    elseif NumComponents > 1 % Have more then 1 object
        TempDist2Center = [];
        for k = 1:NumComponents
            TempDist2Center(k) =
pdist2(ImgCenter,MaskedObjArea{ImgNum}(k).Centroid);
        end
        [~, MInd]= min(TempDist2Center);
        ObjAreaSeries(ImgNum,:) = MaskedObjArea{ImgNum}(MInd).Area;
        Archive.MaskedBounds{ImgNum,1} =
Archive.AllMaskedBounds{ImgNum}{MInd};

        ExtremPts{ImgNum} = MaskedObjArea{ImgNum}(MInd).Extrema;
    else
        ObjAreaSeries(ImgNum,:) = MaskedObjArea{ImgNum}.Area;
        Archive.MaskedBounds{ImgNum,1} =
Archive.AllMaskedBounds{ImgNum}{1};

        ExtremPts{ImgNum} = MaskedObjArea{ImgNum}.Extrema;
    end
    ExtrPtsOrig = ExtremPts{ImgNum};
    %Check Orientation
    if pdist2(ExtrPtsOrig(1,:),[0,0]) <
pdist2(ExtrPtsOrig(1,:),[size(IMGData_Doub,1),0])
        ExAct = ExtrPtsOrig([1 3 5 7],:);
    else
        ExAct = ExtrPtsOrig([2 4 6 8],:);
    end
    CDP = pdist2(ExAct,ExAct);
    % Assume Extrema go in order (clockwise or counter clockwise,
doesn't matter)
    TempC = CDP([2,4,7,12]); % Index style since easier
    TempAv = mean(TempC(:));
    TempSt = std(TempC(:));
    ExtremPts{ImgNum} = ExAct;
    ClustPtsDist = TempC';
    % Temporary adjustments here in case the hydrogel curls. Need
something
    % better because this isn't quite right?
    if TempSt > 125 %125/30.42 % Assume curling is going on,
arbitrary number here for numerator
```

```
        Archive.ClustPtsArch.AvgClustPtsDist(ImgNum,1) =
max(ClustPtsDist) * 30.42;
        isCurledIndicator(ImgNum) = 1;
    else
        Archive.ClustPtsArch.AvgClustPtsDist(ImgNum,1) =
mean(ClustPtsDist) * 30.42;
    end
    Archive.ClustPtsArch.StdClustPtsDist(ImgNum,1) =
std(ClustPtsDist) * 30.42;
    Archive.ClustPtsArch.ClusterDist{ImgNum,1} = ClustPtsDist;
    Archive.ClustPtsArch.ExtrPts{ImgNum,1} = ExtremPts{ImgNum};
    Archive.ClustPtsArch.ExtrPtsOrig{ImgNum,1} = ExtrPtsOrig;

end
% Convert total number of pixels to square microns
% CalibObjAreas = ObjAreaSeries/(0.9/4)^2;% Microscope is 0.9px/um
CalibObjAreas = ObjAreaSeries*(30.42)^2; % GelImg is 30.42 um/px
(1/25/18)
CalibSideLengths = Archive.ClustPtsArch.AvgClustPtsDist;
Archive.ClustPtsArch.isItCurled = isCurledIndicator;

%% Save the Data:
close all;
SlashInds = regexp(Pathname,'\');

SaveDir = Pathname(1:SlashInds(end-1));
save([SaveDir SpecMess 'AreaCalc_' Filename{1}(1:end-4) '_'
num2str(yyyymmdd(datetime))])
% save([SaveDir 'AreaCalc_' Filename(1:end-4)])
disp(Filename{1}(1:end-4));
%% Check plot/results if necessary
NumIms = size(Archive.IMG,3);%num_images;
SpecificImgsToPlot = [];
ImsToPlot = [SpecificImgsToPlot round(linspace(1,NumIms, 8-
numel(SpecificImgsToPlot)))];
BWLineWidth=1;
figure('units','normalized','outerposition',[0 0 1 1]);
set(gcf,'renderer','painters')
for ImageNumber = 1:8
    PlotImNum = ImsToPlot(ImageNumber);
    subplot(2,4,ImageNumber);

imshow(Archive.IMG(:,:,PlotImNum),[min(min(Archive.IMG(:,:,PlotImNum)
)) max(max(Archive.IMG(:,:,PlotImNum)))]);
```

```
    hold on
    ThisBound=Archive.MaskedBounds{PlotImNum};
    plot(ThisBound(:,2),ThisBound(:,1),'g','LineWidth',BWLineWidth);

plot(Archive.ClustPtsArch.ExtrPtsOrig{PlotImNum}(:,1),Archive.ClustPt
sArch.ExtrPtsOrig{PlotImNum}(:,2),'bo','MarkerSize',10,'LineWidth',1.
5)

plot(Archive.ClustPtsArch.ExtrPts{PlotImNum}(:,1),Archive.ClustPtsArc
h.ExtrPts{PlotImNum}(:,2),'rv','MarkerSize',15,'LineWidth',2)
    hold off
    title(num2str(PlotImNum));
end
%% Plot Data
figure('units','normalized','outerposition',[0 0 1 1]);
set(gcf,'renderer','painters')
subplot 121
P1 = plot(CalibObjAreas,'-','linewidth',1.5);
ylabel('Particle Area ({\mu}m^2)')
xlabel('IMG Number')
set(gca,'FontName','Arial','FontSize',20,'FontWeight','Bold')
axis square
subplot 122
P1 = plot((CalibObjAreas-CalibObjAreas(1))./CalibObjAreas(1)*100,'-
','linewidth',1.5);
ylabel('Relative Particle Area (%)')
xlabel('IMG Number')
set(gca,'FontName','Arial','FontSize',20,'FontWeight','Bold')
axis square

figure('units','normalized','outerposition',[0 0 1 1]);
set(gcf,'renderer','painters')
subplot 121
P1 = plot(CalibSideLengths,'-','linewidth',1.5);
ylabel('Side Length ({\mu}m')
xlabel('IMG Number')
set(gca,'FontName','Arial','FontSize',20,'FontWeight','Bold')
axis square
subplot 122

P1 = plot((CalibSideLengths-
CalibSideLengths(1))./CalibSideLengths(1),'-','linewidth',1.5);
ylabel('{\Delta}L/L_0')
xlabel('IMG Number')
```

```
set(gca,'FontName','Arial','FontSize',20,'FontWeight','Bold')
axis square
```

iii. To plot ΔL/L over time

```
% function FluoroIm_PlotDeltaLengthTSer_AveReplShaded()
clear; close all;
% Important parameters:
TimeDiff = 0.5;
ExportPlot = 0;
[Filename,Pathname]=uigetfile('*.mat', 'Select Data to
Compare','C:\Users\jfernace\Desktop\Schulman Lab\Projects\DNA-
crosslinked Hydrogels\','MultiSelect','on');
addpath(Pathname);

Filename=Filename';
%%
MaxCurveLength = 0;
 for WellNum = 1:numel(Filename)

    RawDat=load(Filename{WellNum},'CalibSideLengths','TimeStamps');
    if WellNum>1
        PlottedAreaCurves(:,WellNum) =
zeros(max(size(PlottedAreaCurves,1)),1);
        CurveLength = max(size(RawDat.CalibSideLengths));
        if CurveLength < MaxCurveLength % In case some have more data
points than others, like during mid-expt analysis
            MaxCurveLength = CurveLength;
        end
    else
        MaxCurveLength = max(size(RawDat.CalibSideLengths));
    end
    % Gather the area over time
    PlottedAreaCurves(:,WellNum) = RawDat.CalibSideLengths;

end
PlottedAreaCurves = PlottedAreaCurves(1:MaxCurveLength,:);

for k = 1:WellNum
    DeltaSwelling(:,k) = (PlottedAreaCurves(:,k)-
PlottedAreaCurves(1,k))./(PlottedAreaCurves(1,k));
end
% TimeStamps =
```

```
linspace(0,size(PlottedAreaCurves,1)*TimeDiff,size(PlottedAreaCurves,
1));
TimeStamps = RawDat.TimeStamps;
%% Plot Data
% 15 1:2 3:5 6:8 9:11 12:14 DataToPlot = [   ];
DataToPlot = {[];[]};%[1 2 3 10];[4 5 6];[8];[11];[12]{[1 6 11];[2 7
12];[3 8 13];[4 9 14];[5 10];[16];[17];[18];[19];[15 20]}[4 5 6];[7 8
9]; [10 11 12];[13 14 15];[16 17 18][1 2 3];[4 5 6];[7 8 9];[10 11
12]
%[1 2 4];[7 8];[9 10]
% DataToPlot = {[2 3];
%     [4:6];
%     [7:9];
%     [10 12]};
PlotTimePointRange = [1:numel(TimeStamps)];
CCounter =1;
TempDeltaAve = [];
for i = 1:numel(DataToPlot)
    DTP = DataToPlot{i};
    TempDelta=[];
    for Ti = 1:numel(DTP)
        TempDelta(:,Ti) = DeltaSwelling(PlotTimePointRange,DTP(Ti));
    end

%     TempData{i} =
PlottedAreaCurves(PlotTimePointRange,DataToPlot{i});
    TempDeltaAve(:,CCounter) = smooth(mean(TempDelta,2),5);
    TempDelSTES(:,CCounter) =
smooth(std(TempDelta,[],2)/sqrt(numel(DTP)),5);% Standard Error of
the Mean
    CCounter = CCounter+1;
end
TempUpperBounds=
TempDeltaAve+TempDelSTES;%TempDeltaAve+TempDelSTES*1.96
TempLowerBounds=TempDeltaAve-TempDelSTES;%95% confidence intervals
% TempData = [];
% TempDelta = [];
% for i = 1:numel(DataToPlot)
%     TempData{i} =
PlottedAreaCurves(PlotTimePointRange,DataToPlot{i});
% end
 TempTimeStamps = TimeStamps(PlotTimePointRange);
 TempTimeStamps = TempTimeStamps-TempTimeStamps(1);
```

```
LineStyles = {'-';':';'--'};
BlueSpace = jet(numel(TempDeltaAve));
% LegendText = {'expanded/fresh HPs';'expanded/reused HPs';'no
expansion/heated';'no heat'};
LegendText = {'1','2','3'};   %S5 PEGDA20k+1%term+40uM','S5
PEGDA20k+0%term+40uM','S5 PEGDA100k+1%term+40uM','S5
PEGDA10k+0%term+40uM','S5 Am5mMBIS+1%term+40uM','S5
PEGDA20k+1%term+60uM','S5 PEGDA20k+0%term+60uM','S5
PEGDA10k+1%term+60uM','S5 PEGDA10k+0%term+60uM','S5
Am5mMBIS+2%term+40uM'};
%'Right HPs in SPSC';'Right HPs in PBS';'Right HPs in TAE/12.5mM
Mg++';'Right HPs in TAE/100mM Na+';
%'50% Term HPs';'Different Sys HPs''20 {\mu}M
HPs''0.1xSPSC';'0.2xSPSC';'0.5xSPSC';'1xSPSC';'2xSPSC'
%'2% Term HPs';'5% Term HPs';'10% Term HPs';'50% Term
%HPs';'PEGDA10k';'PEGDA6k';'PEGDA575';'Different Sys HPs';'20 {\mu}M
HPs SPSC';'no HPs SPSC';'40 {\mu}M PolyTs';'20 {\mu}M HPs TAEM''0%
Term HPs 1xSPSC';'2% Term HPs 1xSPSC';'5% Term HPs 1xSPSC';'10% Term
HPs 1xSPSC';'no HPs 1xSPSC';'Right HPs in TAE/12.5mM Mg++';'Wrong HPs
in TAE/12.5mM Mg++';'Right HPs in SPSC';'Wrong HPs in SPSC';'no HPs
in SPSC';
BaseName = ['S6'];
CMap = jet(numel(DataToPlot)*2);
% ExportPlot=1;
%%
fhand=figure('units','normalized','outerposition',[0 0 1 1]);
set(gcf,'renderer','painters')
hold on;
PUpper = plot(TempTimeStamps,TempUpperBounds,'--
','linewidth',1,'Color',[0.65 0 0.3]);
PLower = plot(TempTimeStamps,TempLowerBounds,'--
','linewidth',1,'Color',[0.65 0 0.3]);
%for WellNum =1:numel(PLower) % Convert this to patch if need be
    %fill([TempTimeStamps';flipud(TempTimeStamps')],[TempUpperBounds(
:,WellNum);flipud(TempLowerBounds(:,WellNum))],CMap(2*WellNum-
1,:),'EdgeColor','none','FaceAlpha',0.05);
%end
%yyaxis left
P1 = plot(TempTimeStamps, TempDeltaAve,'-','linewidth',3.5);
for WellNum = 1:numel(P1)
    P1(WellNum).Color = CMap(2*WellNum-1,:);
    PUpper(WellNum).Color = CMap(2*WellNum-1,:);
    PLower(WellNum).Color = CMap(2*WellNum-1,:);
end
```

```
ylabel('{\Delta}L/L_0')
xlabel('time (hrs)')
legend(P1,LegendText,'location','southeast')
xlim([0,100])
% set(gca,'xtick',linspace(0,30,7));
ylim([0 1.4]); %ylim([-0.05 1]);


set(gca,'FontName','Arial','FontSize',26,'FontWeight','Bold')
axis square
box off;hold off
%%
if ExportPlot
    BasePath = Pathname;
    addpath('C:\Users\jfernace\Documents\MATLAB\User Submitted
codes\export_fig_v12122016');
    export_fig_JF([BasePath BaseName '_avgs_'
num2str(yyyymmdd(datetime))],'-svg','-m1','-painters','-
transparent','-q101',fhand);
    export_fig_JF([BasePath BaseName '_avgs_'
num2str(yyyymmdd(datetime))],'-png','-m1','-painters','-
transparent',fhand);
    rmpath('C:\Users\jfernace\Documents\MATLAB\User Submitted
codes\export_fig_v12122016');
    %     close gcf
end
```